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Effects of Inactivity on Cardio-Metabolic Responses to Exercise

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Effects of Inactivity on Cardio-Metabolic Responses to Exercise

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Dedication

To my wife, Cassady. For your unending love and support without which none of this would be possible.

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Abstract

Effects of Inactivity on Cardio-Metabolic Responses to Exercise

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Physical inactivity has been known to cause deleterious health effects. New evidence suggests current physical activity recommendations may not be enough to reduce the risk of developing cardiovascular disease and mortality for those experiencing high levels of physical inactivity (e.g.; prolonged sitting). The purpose of study one was to determine if daily physical inactivity in a group taking low steps (i.e.; 4,767±377 steps/day, LS) impairs postprandial lipemia (PPL), fat oxidation, and submaximal exercise responses to short term training, compared to a group taking high steps (16,048±725 steps/day; HS). After an initial high fat tolerance test (HFTT) to establish baseline responses to a high fat meal, participants (n=16) completed an 11-day training program with assigned step counts and five exercise training bouts consisting of 20 minutes of cycling at 80% VO_{2peak} and two 5-minute intervals at 90% VO_{2peak}. The day following the first and final bouts of exercise training, participants completed a second and third HFTT, respectively, to assess acute responses of PPL to the training. Within HS, a 31% reduction ($p<0.05$) was observed

in plasma triglyceride incremental area under the curve (AUCI) after acute, as well as a 27% reduction ($p<0.05$) following chronic training. Further in HS, but not LS, there were significant ($p<0.05$) reductions in markers of stress during submaximal exercise, such as blood lactate and heart rate, after training. These findings suggest step reductions can lead to an impaired ability to adapt to short term exercise training. The purpose of study two was to determine the effect of reducing step count over two days on the ability of a 1-h bout of exercise to reduce PPL. Participants (n= 10) completed three trials: Low (2,675±314 steps/day), Limited (4,759±276 steps/day) and Normal Activity (8,481±581 steps/day) for two days followed by a 1-h bout of treadmill running at 64% VO_{2max} with a HFTT the following morning. PPL responses following 2,675 and 4,759 step/day trials did not differ. However, following exercise in a condition of 8,481 steps/day, AUCI was reduced 22% and 23% ($p<0.05$) compared to the 2,675 and 4,759 step/day trials, respectively. This suggests that a 1-h bout of running has a decreased ability to lower PPL the next day when taking 4,759 steps/day or less. Taken together these studies highlight the importance of maintaining a healthy level of daily non-exercise physical activity, regardless of participation in exercise. From these studies it is recommended that individuals maintain a daily step count of at least 8,500 steps in additional to any planned exercise in order to achieve improvements in PPL as a result of acute or chronic exercise.

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Chapter I: General Introduction

The consequences of physical inactivity have been recognized for four millennia or more (19, 20, 112) and the evidence for health benefits of physical activity have been clearly documented (68, 203, 208). Nevertheless, physical inactivity continues to be a growing problem for the health and wellness of large swaths of the population worldwide (105). Physical activity has been almost systematically engineered out of the daily lives of many, with advancements in automation and mechanical transport, drastically increasing the amounts sedentary time and inactivity overall (9, 26). The prevalence and effects of physical inactivity on health result in an impact on the population that, while not given the same attention, is at least as deleterious as that of smoking and obesity (112). However drastic the consequence, there seems to be no abatement of this trend in sight (65).

From the work of Morris (133) through present day investigations physical inactivity has been directly linked to at least 35 chronic diseases directly linked to physical inactivity (20). Of note in the diseases linked to physical inactivity is atherosclerotic cardiovascular disease, which remains the leading cause of both death and disability worldwide (6, 78). In 1979, Zilversmit et al. (223) characterized atherosclerosis as a postprandial phenomenon. The postprandial or non-fasting state predominates the waking hours of individuals in most developed countries, whose population rarely go more than 6-8 hours without eating a meal. A rise in physical inactivity in these countries leads to scenario in which these two regularly coincide and may further amplify the elevation of plasma triglycerides which typically peaks 3-5 hours after a meal. If these plasma

triglycerides remain abnormally elevated the result is the deposition of fatty plaques in the arterial walls which characterizes atherosclerosis (223).

Current recommendations advise combatting the development of cardiovascular diseases, like atherosclerosis, with 30 minutes of moderate-to-vigorous physical activity a day or 150 minutes per week (149, 157, 208). Indeed, available evidence clearly documents the ability of a single bout of exercise to attenuate the exaggerated rise is plasma triglycerides following a meal, or postprandial lipemia (PPL), in individuals who are physically active and who normolipidimic (52, 77, 83, 120) and hyperlipidemic (124, 221) and across a range of training status (54, 66, 77). Although these well-established (49) and newly formed recommendations (39) for exercise are firmly grounded in data driven conclusions, it seems some individuals who achieve levels of activity commensurate with these recommendations are not incurring the protective effects of said activity (1, 16, 98, 150, 198, 218). In several recent studies, inactivity has been shown to prevent individuals from realizing classic improvements in postprandial triglyceride (1, 98) and glucose (38) metabolism following a bout of exercise. Similar impairments have also been shown to occur in protein synthesis following prolonged inactivity (22).

This alarming phenomenon, which has been termed ‘exercise resistance’(98), has just begun to be examined and warrants much more investigation. These studies indicate that efficacy of exercise, per se, may be diminished or even abolished if it does not coincide with a healthy lifestyle characterized by daily physical activity. Therefore, it is important to carefully control levels of daily physical activity when examining the protective effect of exercise on PPL. While previous investigations have evaluated the response to a single

bout of exercise, there are no studies that have investigated the effect of more regular exercise bouts over a few weeks, in conjunction with a physically inactive lifestyle. Further, the current literature does not clearly delineate at what levels of physical inactivity this phenomenon becomes significant. In these studies, we examined 1) the effect of an accumulated training stimulus (i.e. 5 training bouts) on PPL and other classic responses to exercise and 2) the PPL response to exercise across three levels physical inactivity.

Chapter II: Purpose and Hypothesis

STUDY #1: Study 1 focused on the adaptations to 5 bouts of exercise training at high intensities in two separate groups with a low and a high level of physical activity. The purpose of study 1 was to determine if the background level of daily physical inactivity (e.g.; ~4,767 steps/day) impairs postprandial lipemia (PPL) compared to an active control condition (e.g.; 16,048 steps/day) and to determine if the background level of daily physical activity impairs other cardiovascular and metabolic adaptations to short term training compared to an active control condition. We hypothesized that a low daily step count treatment would lead to a lower responsiveness to the exercise training compared with a high step count group. The measurements made include the area under the curve for plasma triglyceride (PPL) and fat oxidation in response to a high fat meal, as well as post-intervention measures of peak oxygen consumption (VO_{2peak}) and submaximal heart rate, blood lactate concentration and muscle deoxygenation.

STUDY #2: Study 2 focused on the effect of inactivity, measured by daily step count for 2 days on the ability of acute 1-h bout of moderate intensity exercise to reduce the postprandial plasma triglyceride and plasma glucose responses to a meal high in fat and glucose. The purpose of study 2 was to determine the effect of altering daily step counts for two days (i.e.; 2,675, 4,759, and 8,481 steps/day) on the ability of a 1-h bout of moderate-intensity exercise to reduce PPL. We hypothesized that increasing daily step counts would lead to greater reductions in area under the curve for plasma triglyceride in

response to a high fat meal as well increases in fat oxidation, in response to acute exercise. This pattern might allow recommendations about the number of steps per day needed to achieve a healthy PPL and fat oxidation responses following a high fat meal.

Chapter III: Study #1

THE EFFECT OF PROLONGED SITTING ON CARDIO-METABOLIC RESPONSES TO SHORT TERM EXERCISE TRAINING

Abstract

Background: The effects of exercise and physical inactivity on development of cardiovascular disease have been evaluated individually in numerous investigations. Yet in reality the two interact but the concurrent effects have yet to be fully described. Therefore, the objective of the current study was to investigate in young adults, whether an inactive group (<5,000 steps/day) responds similarly to short-term aerobic exercise training compared to a highly active group (>15,000 steps/day)

Methods: Sixteen initially sedentary participants completed an intense short-term training protocol while taking 4,767±377 steps/day (n=8) or 16,048±725 steps/day (n=8). Participants completed five bouts of training at ~79% VO_{2peak}. Following acute exercise and short-term training, metabolic responses to a high fat meal (i.e. plasma triglyceride and glucose excursions and areas under the curve and fat oxidation) were assessed during a 6-hour high fat tolerance test (HFTT) on the morning after exercise and compared to a non-exercise baseline HFTT completed before the initiation of the training. Additionally, submaximal exercise responses were recorded during 15-minute cycling test (~79% VO_{2peak}), including: heart rate, blood lactate, and deoxygenated hemoglobin were compared within and between groups, before and after training.

Results: Maintaining 16,048 steps/day while completing short-term exercise training resulted in reduced incremental area under the curve (AUCI) for plasma triglyceride concentrations by 31% after acute exercise and by 27% after chronic training, compared to baseline ($p<0.05$). This was accompanied by increased whole-body fat oxidation ($p<0.05$). Further, muscle stress during submaximal exercise, as marked by heart rate, blood lactate

and deoxygenated hemoglobin, was also reduced ($p<0.05$). Despite completing the same training regimen, participants taking <5,000 steps/day showed no significant improvements in postprandial responses or markers of stress during submaximal exercise after training ($p>0.05$). However, the two groups showed a similar increase in VO2peak.

Conclusion: In conclusion, when completing a 5-bout exercise training program at vigorous intensity, decreasing daily steps to approximately 5,000 steps/day appears to prevent or significantly blunt some of the classic cardio-metabolic adaptations that occur with 16,000 steps/day. Based on these findings, it appears that the effects of inactivity cause a blunting of the normal adaptations to exercise including both cardio-metabolic measures as well as exercise stress measures such as heart rate, blood lactate concentrations, and deoxygenation of hemoglobin in the active muscle, but not VO2peak.

Introduction

The modernization of society has led to vast technological advancements that have changed life throughout the world. These advances have intended to improve the quality of life for many individuals, and to a degree, increased automation has done so. However, the resultant increased physical sedentary time in modernized cultures has been accompanied with serious problems. Public health professionals assert that individuals in modern society have become a victim of their own success, in which physical activity have been systematically "engineered out" of daily life, and further argue that this has compromised the health of many (100).

Couple this with improvements in agriculture and sustainability and people are spending more time being inactive in a fed state. Ingestion of fatty foods causes a concomitant rise in chylomicron triglyceride concentration in the blood as lipids from digestion begin to enter the blood and are subsequently cleared into other tissues over the course of up to 8-12 h (32, 56, 86). The rise in chylomicron triglyceride in the blood after a meal high in fat produces postprandial lipemia (PPL). The high concentration of triglycerides in the plasma leads to subsequent breakdown into more atherosclerotic byproducts, resulting in the possible formation of atherosclerotic plaques in arteries (135). Along with the rise in sedentary behavior has come an increase in the mortality rate as caused by cardiovascular disease (137). As early as 1979, atherosclerosis was described as a "postprandial phenomenon" (223). Exaggerated rises plasma triglyceride concentration

after a meal (32, 56, 86) caused by a variety of factors including extended periods of inactivity (3, 33, 51, 135) may be driving the pathogenesis of atherosclerosis.

Exercise has been a well-documented method to attenuate the rise in PPL (76, 83, 120, 168, 222) and prevent a detriment to cardiovascular function (27, 50, 61, 95, 160, 200). However, recent epidemiological evidence suggest that exercise training may not be adequate to reduce incidences of disease and other morbidities, and even death in people who spend a large amount of time sitting (70). Despite well-established (49) and newly formed recommendations (39), exercise performed concomitant with these recommendations may not be enough to overcome the detrimental effects of sedentary time.

New evidence has emerged suggesting prolonged inactivity and sedentary time may impede or eliminate the positive effects classically associated with exercise (38, 98). In an investigation by Kim et al (98) a group of individuals who sat for >14 h/day for 4 days did not show the "classic" attenuation PPL that has been shown previously with 60 minutes of aerobic exercise on the fifth day (99, 183). These authors termed this phenomenon "exercise resistance" because it seems exercise was unable to acutely improve cardio-metabolic indicators of health (e.g.; PPL and relative fat oxidation). In order to counteract this "exercise resistance" due to prolonged sitting higher levels of physical activity throughout the day may be needed. In fact, some studies are now pointing to the idea that breaking up sedentary time, independent of total moderate to vigorous physical activity, may be able to attenuate PPL (38, 69, 153) and restore or maintain endothelial function (127, 132, 176). It is also important to systematically

evaluate if this "exercise resistance" extends to other typical training adaptations besides improved lipid tolerance.

Most of these studies have evaluated the effect of inactivity time on responses to acute (e.g.; one bout) of exercise. Yet, to test a true "exercise resistance" phenomenon it would be vital to assess training adaptations incurred over the periods of training than longer than simply an acute exercise bout. Thus, it is imperative to determine if short-term training adaptations (i.e.; after 5 bouts of exercise training over 9 days) are blunted in participants who are also relatively inactive outside of training (i.e.; <5,000 daily steps).

The purpose of this study is to determine if cardiovascular and metabolic responses to exercise are improved in individuals who participate in intense exercise training, yet reduce daily steps below 5,000, over the course of 5 training bouts over 9 days compared to a group following the same training protocol but are physically active (i.e.; >15,000 daily steps). We proposed examining the differences in physiological (as assessed via heart rate, blood lactate, and NIRS) and metabolic (as assessed by postprandial triglyceride and glucose responses) adaptations to short-term training between Low Step (LS) and High Step (HS) treatments. We hypothesized LS would exhibit differences in both physiological and metabolic adaptations to short-term training compared to HS.

Methods

Sixteen healthy, initially sedentary and untrained male (n= 8) and female (n=8) participants were recruited and randomly assigned to two groups. Both groups completed a training regimen administered under supervision of the investigators. Outside of said exercise regimen, one group was physically active (n=8), taking 16,048 steps/day, and the

other group was sedentary (n=8), taking 4,767 steps/day. Both groups were asked to refrain from any planned exercise outside of the experimental design. Participants were given written and verbal description of all the procedures and measurements used in this study, and written informed consent was obtained. The Institutional Review Board of the University of Texas at Austin approved this study (ClinicalTrials.gov Identifier: NCT03352063).

Experimental Design

The experimental design consisted 17 days with three distinct phases (see Figure 1). Days 1-3 (Pre-training) consisted of baseline or pre-training measures. Days 4-14 (Training) consisted of alternating days of training and rest days. The final three days, 15-17, (Post-Training) consisted of repeating measurements taken in pre-testing phase.

Following informed consent and completion of a health history questionnaire, On the first day participants visited the Human Performance Laboratory (HPL) for initial or baseline high fat tolerance test (HFTT). One the second day determination of peak oxygen uptake while cycling (VO_{2peak}). The following day, D3, participants completed a 15-minute submaximal cycling test at 79% of VO_{2peak} value. While the maximal and submaximal tests were being conducted, participants wore the activity monitor for familiarization purposes. This activity monitor (activPAL, PAL Technologies, Glasgow, Scotland)) is small and noninvasive in nature, measuring roughly 2 in x 1in x 0.1in in size and worn anteriorly on the thigh. The monitor was placed in a small rubber sheath and

attached via transparent film dressing. The activity monitor is not waterproof and cannot be worn while showering. Participants were thus instructed to remove the device prior to showering and were provided with the materials to change the dressing immediately after showering once the area is dry. Therefore, aside from showering, the activity monitor was worn continuously throughout the training phase (D4 - D14). After testing on D3, participants were asked to refrain from any planned exercise and to begin adhering to the prescribed daily step count.

After the first bout of exercise training on the evening of day 6, another HFTT was performed on day 7 to evaluate responses an acute bout of exercise. Participants continued the training regimen, exercising and resting on alternating days such that there were five exercise sessions and four rest days in this training phase. All exercise bouts were identical and consisted of a 20-minute cycling bout at 79% of the participant's pre-training VO_{2peak} followed by 10 minutes of rest. Participants then completed two 5-minute bouts at ~90% VO_{2peak} with 5-minute rest intervals between each bout. This exercise prescription is in line with, or exceeds, the current physical activity guidelines published by the American Heart Association (AHA) and American College of Sports Medicine (ACSM) for improvements in cardiovascular fitness (49).

In the post-training phase, participants completed a HFTT following the final bout of exercise on the evening on D14. On the D15, participants completed another submaximal test at the same duration and absolute work rate as the submaximal test

during the pre-testing phase (15-minute submaximal cycling test at 80% of VO_{2peak}). On the final day participants completed a post-training VO_{2peak} test.

Dietary Control

During the course of the study participants were asked to eat to satiety, following a diet standard in macronutrient breakdown (126). Also, participants logged all food using the MyFitnessPal mobile application (MyFitnessPal, Inc.). Participants were asked to consume the same foods on the day prior to each HFTT. On the evening prior to the HFTT participants were given a low-fat meal to consume as fat in the previous meal can affect the response to a high-fat test meal (42, 184).

High Fat Tolerance Test (HFTT)

On the morning of the HFTT, participants arrived at the HPL following a 12-hour fast and having consumed 500 ml of water 1 hour prior to arrival. Prior to the HFTT, participants have body mass was measured. After resting for 5-minutes, an intravenous catheter was inserted into an antecubital vein. A resting blood sample was taken and 10-minutes later, the HFTT test meal consisting of melted ice cream and heavy cream; approximately 14.8 kcal/kg (0.8 g, 1.2 g, and 0.2 g/kg BW of carbohydrate, fat, and protein, respectively) was consumed in 5-minutes. Blood samples were then taken hourly for the next 6-hours.

Postprandial Substrate Oxidation

During the HFTT, expired gas was collected for determination of whole body carbohydrate and lipid metabolism. Participants rested for 10-minutes in a seated position, followed by 10-minutes of expired gas collection via meteorological balloons performed at 0, 2, 4, and 6 hours. It has been previously demonstrated that inactivity reduces whole body fat oxidation (98).

Energy expenditure and substrate oxidation were calculated from oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER), energy expenditure and substrate oxidation were calculated based on the methods of Lusk (118), below.

% Energy from carbohydrate (CHO) oxidation = ((RER – 0.707)/0.293) x 100

% Energy from fat oxidation = 100 – % Energy from CHO oxidation

CHO oxidation (kcal/min) = (%CHO oxidation/100) x VO_2) x 5.05kcal/L O_2

Fat oxidation (kcal/min) = ((1-%CHO oxidation/100) x VO_2) x 4.7kcal/L O_2

Energy expenditure (kcal/min) = CHO oxidation + Fat oxidation

Maximal Oxygen Consumption Testing

During this procedure, participants breathed into a mouth-piece (while wearing a nose-clip) that collected and analyzed the O_2 and CO_2 content of expired air. From this participants oxygen consumption was determined and their peak value (VO_{2peak})

identified. The intensity of exercise, measured in watts, was increased every 1-2 min. until they reached their maximal effort level and become fatigued. Volitional fatigue was associated with a difficulty or inability to maintain cadence (>60 RPMs) while cycling. The total length of the test was ~6-12 min, including a 4-minute warm-up. Heart rate was also measured continuously from a strap worn around the chest (Suunto, Vantaa, Finland). Heart rate data was used as a validation method for obtaining VO_{2peak}.

Submaximal Exercise Testing

Submaximal exercise testing was conducted on a cycle ergometer and consisted of a 15-minute bout at an intensity of ~80% of VO_{2peak} derived from the VO_{2peak} testing described above. Blood samples were taken from an indwelling venous catheter at the beginning and end of the 15 min submaximal exercise protocol to evaluate blood lactate responses. Heart rate and VO_2 were measured continuously, as described above. Near-Infrared Spectroscopy (NIRS) (OxiplexTS, ISS Oximeter Model 95205, Champaign, IL) was used to measure deoxygenated hemoglobin during exercise in the vastus lateralis, as a final measure of physiological stress during submaximal testing. The acquisition frequency of 2 Hz was used for this study. The data between 9 and 10 minute of the testing protocol were averaged and recorded.

Biochemical Analysis

For plasma triglyceride and glucose concentrations, all blood samples collected

were immediately transferred to K_2 EDTA collection tubes (BD Vacutainer, Franklin Lakes, NJ), centrifuged at 3,000 g for 15 minutes at 4◦C. Plasma was then stored in separate aliquots at -80◦C until later analysis. All measurements for each participant were performed in duplicate within the same analysis. Plasma triglyceride and glucose concentrations were measured by a spectrophotometric method using commercially available kits (Pointe Scientific, Inc. Canton, USA).

For blood lactate concentrations, Blood samples were immediately deproteinized by placing it in 8% perchloric acid and lactic acid levels were later measured on the supernatant. Enzymatic analysis was used to determine blood lactate concentration based on methods of Farrell et al (41). Intraassay coefficients of variation for plasma triglyceride, glucose, and blood lactate concentrations were all less than 10%.

Statistical Analysis

Descriptive Statistics are reported as Mean ± SE. Descriptive statistics were compared using students t-test (α= 0.05). Differences in daily steps, maximal and submaximal exercise responses, postprandial responses and incremental (AUCI) and total (AUCT) areas under the curve for concentrations of plasma triglyceride and glucose were determined by two-way ANOVA (Treatment X Time). Within group differences in plasma triglyceride concentration and postprandial substrate oxidation were determined using repeated measures two-way ANOVA (Trial X Time). Tukey's LSD was performed to determine if statistical significance exists. All data were analyzed using GraphPad

Prism 7 (GraphPad Software Inc., La Jolla, CA). The probability level for statistical significance was sat at $\alpha = 0.05$.

Results

Participant Characteristics

Participants' characteristics are described in Table 1. The total number of participants was 16 (8 males, 8 females), with each participant randomly assigned to one of the experimental conditions. Participants were generally young (23.6 ± 4.7 years), healthy individuals that were initially sedentary with similar VO_{2peak} values (HS: 34.1 ± 3.3; LS: 32.2 ± 2.9 ml/kg/min, $p>0.05$). There were no differences in age (HS: 23.4. ± 5.6 yrs; LS: 23.8 ± 4.0 yrs), height (HS: 166.4 ± 7.9 cm; LS: 167.2 ± 8.4 cm) or body mass (HS: 74.4 ± 0.1 kg; LS: 72.5 ± 0.2 kg) between groups ($p>0.05$). Participant HR, $\%VO_{2peak}$, RPE during exercise were all similar ($p>0.05$) and suggest exercise bouts that could be classified as vigorous intensity (Table 2).

Daily Steps

Daily Steps (Figure 2) were recorded throughout the experimental design. A significant main effect was found for treatment group. HS treatment group took significantly more daily steps than LS group (HS: 16,048 ± 725 steps/day; LS: 4767 ± 376, $p<0.001$). The groups adhered well to their prescribed step number. However, post hoc analyses revealed that on D7, individuals in HS took significantly less steps than the same group did on D12 and D14 (D7: 11,096 ± 1361 steps/day; D12: 18,524 ± 2481). These differences most likely resulted from HFTT (i.e. required sitting for 6h) that occurred on D7.

Total Plasma Area Under the Curve Responses

Plasma triglyceride concentrations were analyzed at each time point in all trials for both treatments and calculated for incremental area under the curve (AUC_I) and total area under the curve (AUC_T) (Figure 3). Plasma TG AUC_T & AUC_I showed significant interactions (Treatment x Time, $p < 0.05$). Within the LS treatment group, no significant differences were found between HFTT time points at Baseline, Acute or Post-training for the AUC_T or AUC_I. Concurrently in HS, AUC_T was significantly lower in both Acute (760.9 ± 73.7 mg/dL per 6 h, $p < 0.01$) and Post Training (762.2 ± 65.5 mg/dL per 6 h, $p<0.01$) as compared with those in Baseline (886.8 ± 79.6 mg/dL per 6 h) with no significant difference between Acute and Post Training ($p>0.05$). The incremental plasma TG responses (TG AUC_I) was significantly different in both Acute (221.7 ± 49.7 mg/dL per 6h, $p<0.05$) and Post Training (236.7 ± 61.4 mg/dL per 6h, $p<0.05$) compared to Baseline AUC_I (322.9 ± 67.2). Additionally, no differences were detected between Acute and Post Training AUC_I. Furthermore, plasma glucose AUC_T and AUC_I showed no significant effects within, or between either treatment groups ($p > 0.05$) (Figure 4). Overall, no between group differences reach statistical at Baseline, Acute or Post-Training ($p>0.05$).

Postprandial Substrate Oxidation

Postprandial substrate oxidation was determined using indirect calorimetry (Table 3). Oxidation calculations were limited to 7 participants from each treatment group due to possible hyperventilation at rest. Evaluation of postprandial RER data revealed significant differences. Within HS, RERs during the HFTT were reduced after both Acute (0.79 ± 0.01) and Post Training (0.80 ± 0.01) compared to Baseline (0.83 ± 0.01, $p > 0.05$). However, but no significant differences were found within LS or between treatment groups. Likewise, Percent carbohydrate oxidation and percent fat oxidation were found to have significant differences between trials in HS ($p<0.05$); while no differences were seen within LS or between trials. Further, postprandial absolute fat oxidation (i.e.; kcal*6h) was higher by 24% in Acute ($p< 0.05$) and 19% in Post Training ($p< 0.05$) compared with that in Baseline. IN LS, there were no increases in absolute fat oxidation during HFTT between Baseline or Acute or Post Training ($p>0.05$). Finally, energy expenditures for the HFTT were not different within or between trials ($p>0.05$) (Table 3).

Plasma Triglyceride & Glucose Concentrations

Plasma triglyceride and glucose concentrations (Figure 5 & 6) were analyzed at Baseline, Acute and Post-training for both treatment groups and calculated for incremental area under the curve (AUCI) and total area under the curve (AUCT). In LS, no significant difference was found between trials at any time point for the six-hour triglyceride excursion (Figure.6). However, in HS significant differences existed at several time points with Acute and Post Training values compared to Baseline. Hour 1, 2 and 3 measurements were significantly lower in both Acute and Post Training compared with Baseline ($p<0.05$). For

the last two measurements (e.g. H5, H6) of the Post Training HFTT were significantly lower than baseline ($p<0.05$) with no differences between Acute and Baseline or Post Training. No significant differences were found between trials at any time point for the six-hour glucose excursion, between or within either treatment group (Figures 5 & 6).

Exercise Responses

Peak and submaximal exercise responses are summarized in Table 2. Peak oxygen consumption (VO_{2peak}) increased significantly from pre to post training ($p<0.05$). Oxygen consumption and workload during submaximal exercise was similar between groups pre and post training ($p>0.05$) and showed no difference within groups at either time point ($p>0.05$).

Blood lactate concentration increased significantly with exercise ($p<0.05$), while no differences existed between groups at rest ($p>0.05$) or during exercise ($p>0.05$). Pre-intervention data showed no significant differences in HR, RPE, RER between groups ($p>0.05$). After five bouts of exercise training, post-intervention testing revealed significant reductions in HR and blood lactate concentration within HS ($p<0.05$), and no significant changes in LS ($p>0.05$).

Furthermore, NIRS measurements at rest revealed no differences between groups before or after the training ($p>0.05$). After training, deoxygenated Hb (HHb) was significantly lower than pre-testing within the HS group ($p<0.05$). No significant differences were found in LS pre vs post training ($p>0.05$).

Dietary Control

Daily caloric intake and percent of macronutrients were averaged across the 11-day training phase. HS participants consumed 2389.1 ± 153 kcal/day comprised of 50.7 ± 0.3% carbohydrate, 29.8 ± 0.2% fat, & 19.5 ± 0.2% protein. Daily caloric intake for LS averaged 1949.9 ± 47 kcal/day comprised of 51.9 ± 0.4% carbohydrate, 29.2 ± 0.5% fat, & 18.9 ± 0.2% protein. Caloric intake was significantly different between groups ($p<0.001$) No differences existed between groups in percent macronutrient consumption ($p>0.05$).

Discussion

This study investigated the effect of 11 days of inactivity (<5,000 steps/day) on the ability of a short-term exercise training regimen, consisting of 5 bouts over 9 days, to improve postprandial triglyceride and classic markers of training adaptation. The foremost finding was that, against a background of reduced daily steps (i.e.; low step; LS), intense training (i.e.; 20-minute exercise bout at 80% VO_{2peak} with two 5-minute intervals at 95% VO_{2peak}) failed to improve the postprandial metabolic responses to a high-fat meal or promote classic whole body adaptations during submaximal exercise. This is noteworthy, in that exercise of this intensity and duration was found to elicit sizable improvements in high step (HS) as observed previously (46, 124, 183). In those who are active (e.g.; HS), our findings suggest that a single acute bout of the prescribed exercise was effective in lowering postprandial TG. Both AUC_T and AUC_I were significantly lower than baseline after an acute bout of exercise, in the HS group accumulating ~16,000 daily steps. Following four additional bouts of the same exercise postprandial metabolic responses were similar to those seen after the single acute bout. Thus, the exercise was effective at lowering PPL in an active group taking ~16,000 steps/day and additional bouts of training

appear to offer no greater benefit compared to a single bout. This agrees with previous observations suggesting no additive effect of exercise bouts of consecutive days (40, 45).

Therefore, it seems that a reduced daily step count may somehow prevent or severely encumber the healthy cardiometabolic adaptations that normally occur in response to this training, both acute and chronically (46, 76, 77, 81, 83, 120, 221). This inability to derive the protective effects of training, caused by physical inactivity, agrees with the phenomenon of 'exercise resistance' first postulated by Kim et al (98). Further, this study is the first to provide evidence that the implications of exercise resistance may extend to short-term training.

While Kim et al (98) and one other study (1) also induced exercise resistance by reducing daily steps to <2000 and <4000 steps/day, respectively, and used similar HFTT testing procedures, these prior observations were limited to acute exercise bouts. In both these studies a 1-h hour bout of exercise at ~65% VO_{2max} failed to improve postprandial metabolic responses, as would typically be seen following exercise of this duration and intensity (76, 120, 167, 222). The present design contributes significantly in expanding the consequences of daily physical inactivity beyond responses to a single bout of exertion into a paradigm of more regular exercise that would fit within current recommendations (157).

Indeed, epidemiological studies have clearly documented that individuals who meet current recommendations may not realize the reduced risk of CVD usually associated with meeting guidelines, if these individuals are also inactive for the remainder of the day (16, 65). As others have noted (64, 65, 181, 205) it is possible, if not likely, that people living in the US and similar industrialized countries could achieve or exceed physical activity guidelines while still being inactive for 15 or more hours each day. It seems this interplay, of prolonged inactivity and exercise, reduces the potency of the stimulus provided by exercise training (1, 38, 98). However, there is a dearth of data on how the consequences

of this interplay would manifest themselves with even short term training as presently employed. That is to say, while exercise resistance has been shown following acute exercise, one might not expect the same results from a single bout of exercise to be derived from short-term exercise training (96, 157). The findings from this investigation provide evidence explaining, in part, the observations seen in previous epidemiological studies that have found some individuals who are meeting published guidelines on physical activity are not realizing reduced risk of CVD and premature mortality (16, 39, 150, 198, 218).

The findings from this study suggest individuals meeting current physical activity guidelines may not derive the protective effect of daily physical activity to improve health, at least in regard to postprandial triglycerides, if they are also experience extended periods of inactivity. Our finding that, in a group of individuals taking 4767 steps/day, postprandial responses were similar to untrained baseline whether individuals performed a single bout of exercise or five exercise bouts is noteworthy. Further the exercise performed in this study was a higher intensity (i.e. ~80-90% VO_{2peak}) than in previous studies noting exercise resistance (1, 98). This is significant in that higher intensity exercise has been shown to exert greater PPL lowering effects than moderate, or low intensity (99, 183) and may provide additional insight into the potency of the induced 'exercise resistance'.

While our findings did not suggest an additive effect of the final four training bouts for lowering PPL beyond the acute response to exercise, it is important to note that the consistent exercise sessions should not be viewed as ineffective. It is probable that the TG lowering effect of the training was exerted after each additional bout, not just after the first and final bouts when HFTTs were performed, serving to maintain consistently-low daily plasma TG levels. Regular exercise is likely to have beneficial effects on PPL through short-term increases in LPL (164) as well as the other cardiovascular benefits generally (149).

Among these cardiovascular benefits are indicators of muscular stress that are typically reduced following exercise training while exercising at a given intensity. These measures such as heart rate and blood lactate accumulation seem to be similarly unaffected in LS compared to HS. While our findings clearly demonstrate that both conditions (high and low step count) benefited from training, by increasing their VO_{2peak} and thereby decreasing the relative percentage of VO_{2peak} needed to sustain an absolute work rate while cycling, it seems adaptations at the level of the muscle (i.e.; blood lactate) may have been impaired. It is unlikely that the changes seen in HS were due to the higher number of steps causing an additional training effect beyond that provided by the intense training (80-90%VO_{2peak}), but that possibility can't be discounted. Individual who simply increase their daily step count over several weeks, typically don't increase VO_{2max} or show the adaptations to submaximal exercise currently seen in HS (170).This study could have benefitted from an additional control group in which participants maintained a high step count but did not participate in the short-term training. This would have allowed isolation of the effects of increasing daily activity alone. While is it probable that this level of daily walking, in HS, was somewhat higher than the participants would experience during normal daily living, it is highly unlikely that the intensity of walking (e.g. ~30% VO_{2max}) elicited significant adaptation (212). This suggests the lack of improvement in blood lactate in LS pre-vs-post training may be due to inactivity producing an intramuscular environment that might be 'resistant' to the stimulus provided by training.

Reductions in daily steps, whether imposed by prolonged sitting or another form of induced inactivity may lead to a condition in which uptake of substrates in the blood by muscle is reduced. While this requires speculation, it is possible that the similar levels of blood lactate, plasma triglycerides, and plasma glucose where due to decreases in cellular expression or activity of membrane transporters such as MCT, GLUT4, and GPIHBP1

proteins responsible for increased uptake of these energy substrates. Disuse, modeled through denervation of a rats hindlimb resulted in decreases in MCT1 in the soleous and MCT4 in the gastrocnemius (210). Because muscle serves as a primary consumer of lactate during exercise, any decreased expression of MCTs could lead to increased lactate concentrations during steady state exercise, or the lack of improvement blood lactate levels seen in LS. Similarly It has been suggested that hindlimb suspension could result in decreases in GLUT4 expression at the surface of the sarcolemma (94).

Recent observations support this contention in that not only is the postprandial triglyceride response impaired with inactivity but a plethora of other metabolic responses may be diminished or abolished as well (1, 13, 38). Consistent with our findings, Bergouignan et al. (13) showed that 32 days of bed rest increased PPL by 27%, compared to an ambulatory control, and this effect was not averted by exercise training performed every 3 days during the bed rest. Duvivier et al. (38) showed an hour of exercise was not sufficient to counteract the effects of sitting for 13 hours which resulted in no significant improvements in triglycerides, non-HDL cholesterol and apolipoprotein B plasma levels compared to a sitting condition without exercise. Interesting a group with matched energy expenditure through increased daily walking did see improvements in each of those measures, compared to sitting plus exercise, without structured exercise(38). Further reduced myofibrillar protein synthesis can be seen in elderly (22) and young healthy individuals (166) in response to step reductions of two weeks or less. This could be due to reduced uptake of amino acids from circulation, similar to the reduced plasma triglyceride and plasma glucose uptake shown by this study and others (1, 13, 98).

Primary amongst the limitations of the current study was a lack of sufficient power. Power analyses indicated that a sample size of approximately 38 individuals would be necessary to detect between groups differences in triglyceride AUCI. Despite sizable

differences in postprandial triglyceride responses within the respective treatments, it is likely that this lack of power prevented our ability to detect difference between our treatment groups, both in postprandial and exercise responses. Therefore, while it can be concluded that the short term exercise training significantly improved PPL both acutely and chronically in HS, whereas it did not significantly improve PPL in LS, it cannot be concluded that HS was found to be significantly better than LS.

Secondly, it is possible that our results were influenced by selection bias. Participants were all previously sedentary, and were randomly assigned to each 'step' group. It is unclear if that those who participated in the study are fully representative of a broader sedentary population, as they sought out an opportunity to participate in intense exercise testing and training and were willing to commit to and strictly follow a structured program of that nature.

Participants in the study, especially within LS, also began to wane from strict adherence to the daily step protocol as the training phase progressed. Due to the nature of the study, extending 11 days across 2-3 weekends, to carefully ensure adherence to the assigned daily step goal would have caused an undue burden on the participants in this study by removing them from their normal routine. Having participants remain in the laboratory or under direct observation is impractical for the scope of this work. Despite taking more than 6,000 steps/day on a few days prior to the 'Post Training' HFTT (D10, D12, and D13), the relative increase in daily steps seems to have little effect on the postprandial responses. This is evidenced by similar AUCI during 'Acute' and 'Post Training' HFTTs while taking less than 4,400 step/day, on average, in the days preceding the 'Acute' HFTT. This may indicate that 6,000 steps/day is also inadequate to counteract 'exercise resistance' and realize the protective effects of exercise training, at least in terms of PPL. Currently, however, this requires speculation as 'exercise resistance' has only

recently been recognized (98)) and the nature of its development and abatement have yet to be fully described. That is, we cannot say conclusively that 6,000 step/day results in the development exercise resistance in the absence of prior days of an even further reduced daily step count as presented here. Further work is needed to address these issues definitively.

In conclusion, the data presented here suggest that 11 days of step reduction (i.e.; LS; 4,767 steps/day) prevents the improvements in PPL typically seen following intense exercise training when background step count is high (e.g.; HS: 16,048 steps/day). Instead of reductions in TG AUCs, as seen with training in HS, the results indicate no improvements compared to baseline after acute exercise or short-term training. The finding from the current investigation indicate that reducing steps below approximately 5,000 steps/day may generate reduced responsiveness to normal, healthy stimuli of intense short-term exercise training. These findings suggest that reliance on exercise may not be enough to sustain a low PPL in those whose lifestyles are characterized by regular, prolonged inactivity.

Tables and Figures

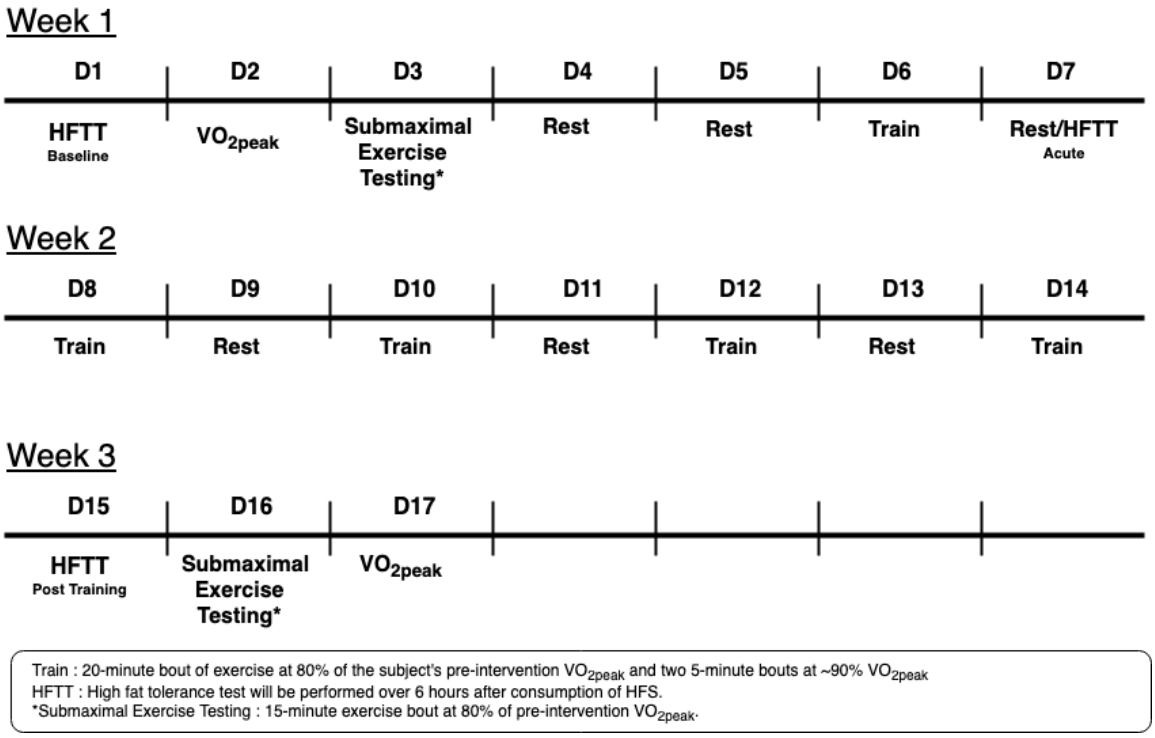


Figure 1. Study Design. Participants were separated into two groups (High Step or Low Step) and completed a short-term exercise regime with physiological and metabolic testing pre and post training. Subjects took their assigned step number on days 4-14.

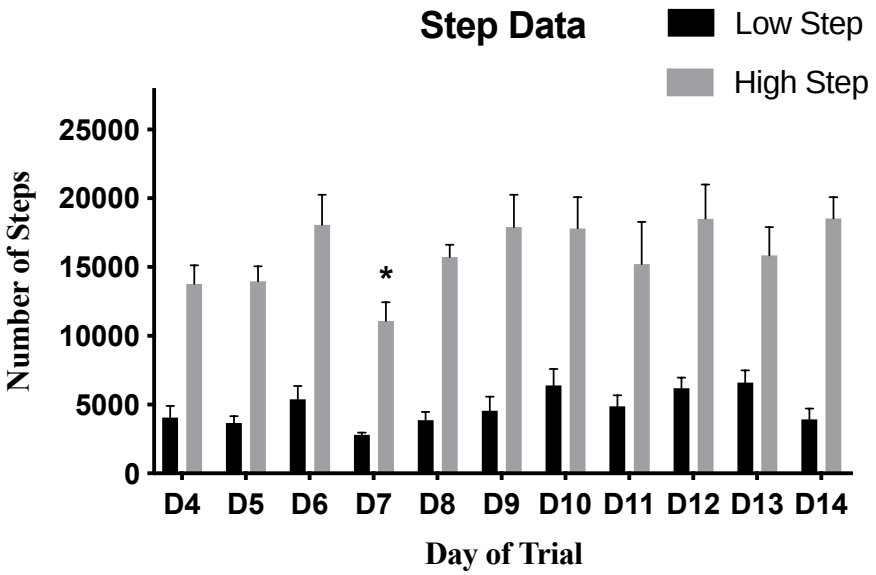
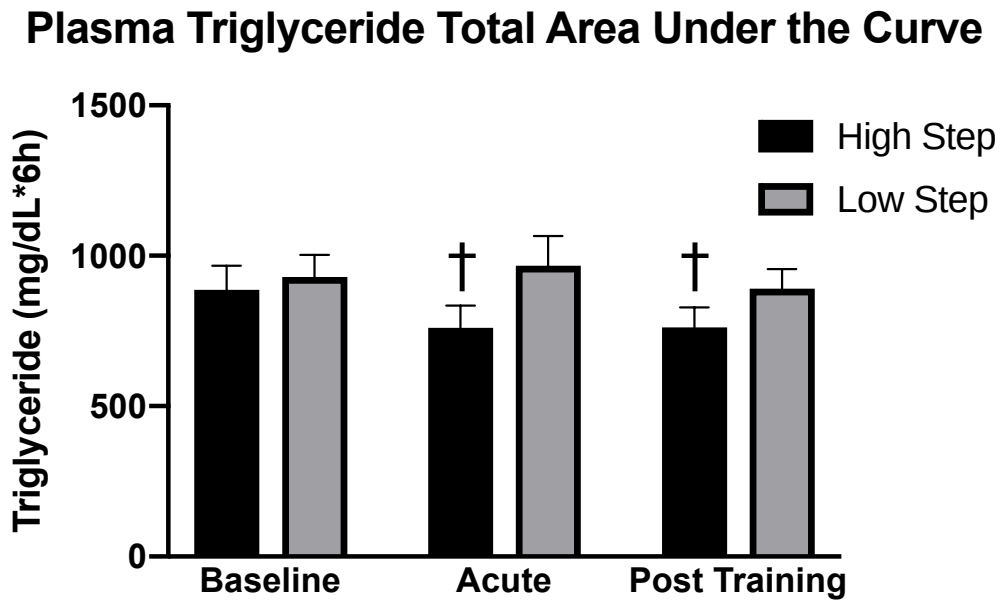


Figure 2. Daily steps were measured via activPal activity monitor, attached on the participant's anterior thigh throughout each trial. Average daily step count, for each group are presented for the 11-day intervention period (D4-D14). Average daily steps were significantly different between groups for every day measured ($p<0.001$). (*) significantly different from D12 and D14 within treatment group ($p<0.05$).



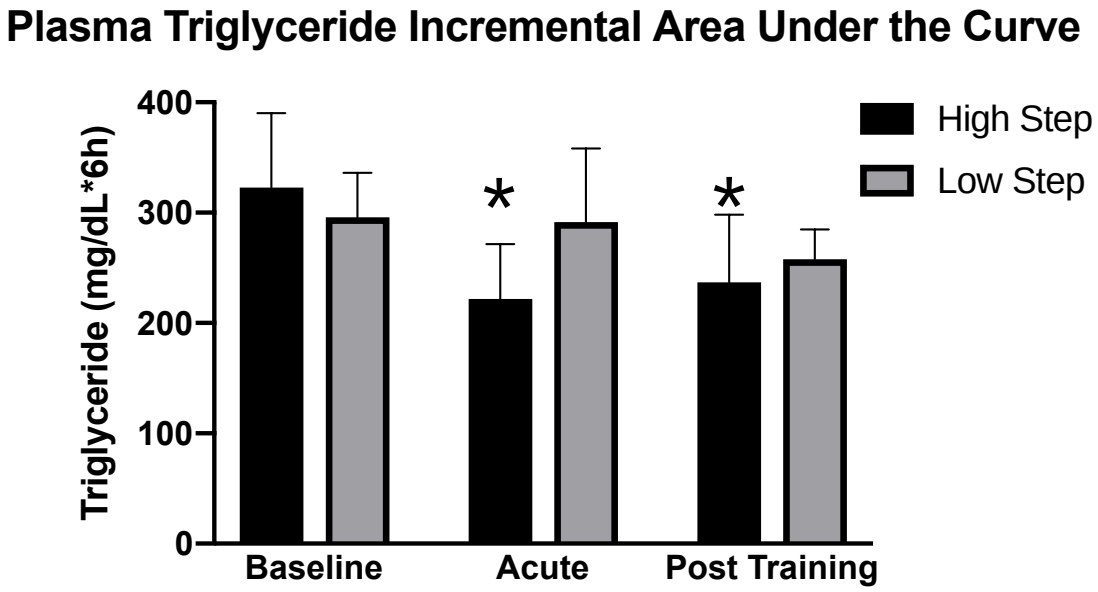
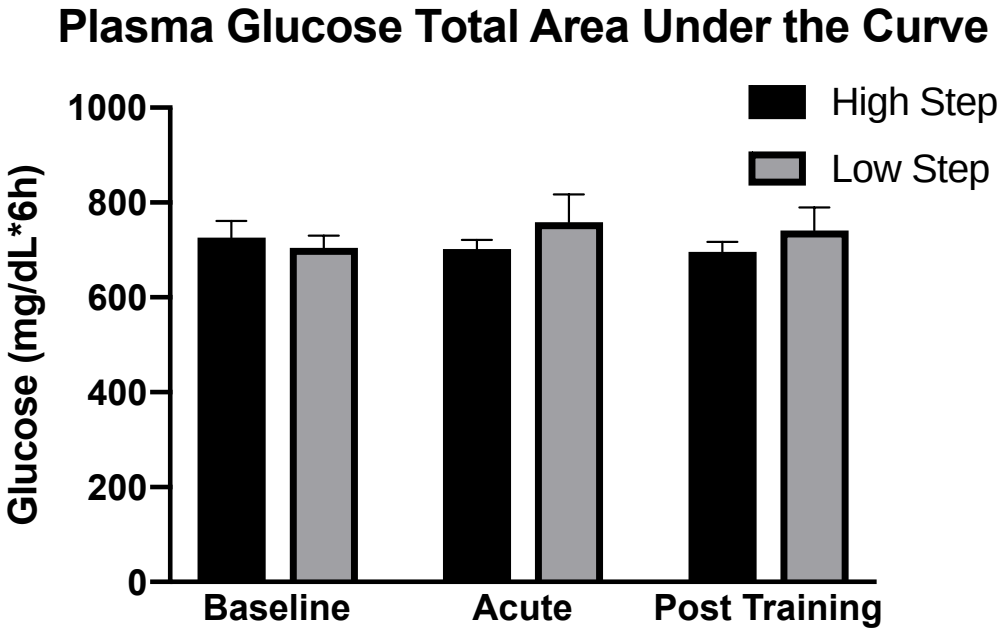


Figure 3. Total and Incremental areas under the curve of plasma triglyceride concentration during HFTT at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). (*) Significantly different from Baseline within treatment group, $p<0.05$. (†) significantly different from Baseline within treatment group, $p<0.01$. Data reported as mean±SE.



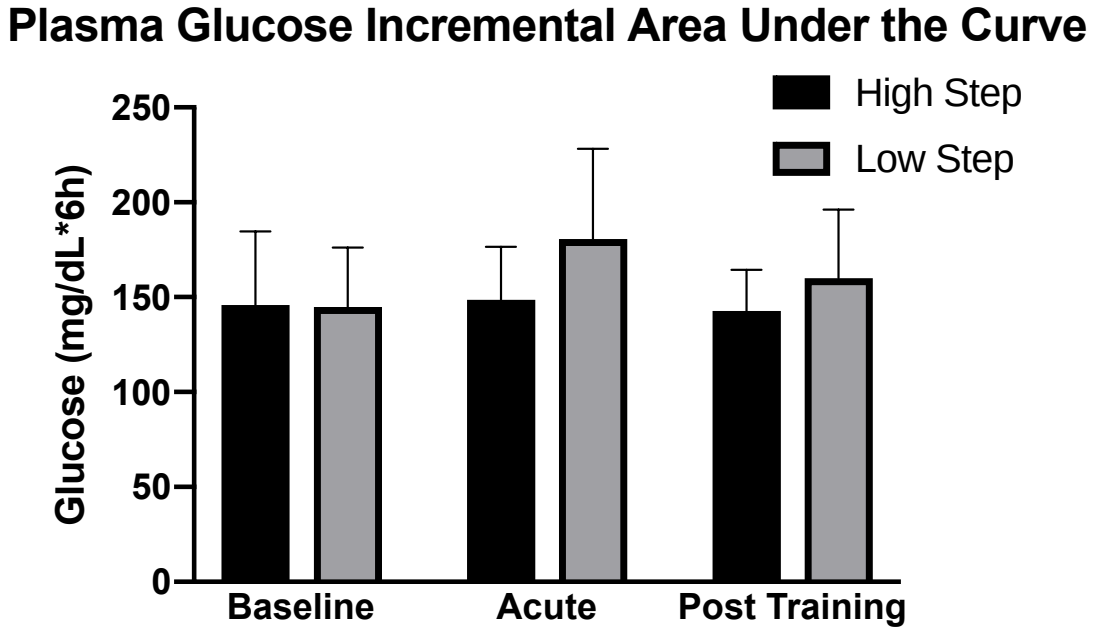


Figure 4. Total and Incremental areas under the curve of plasma glucose concentration during HFTT at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). Data reported as mean±SE.

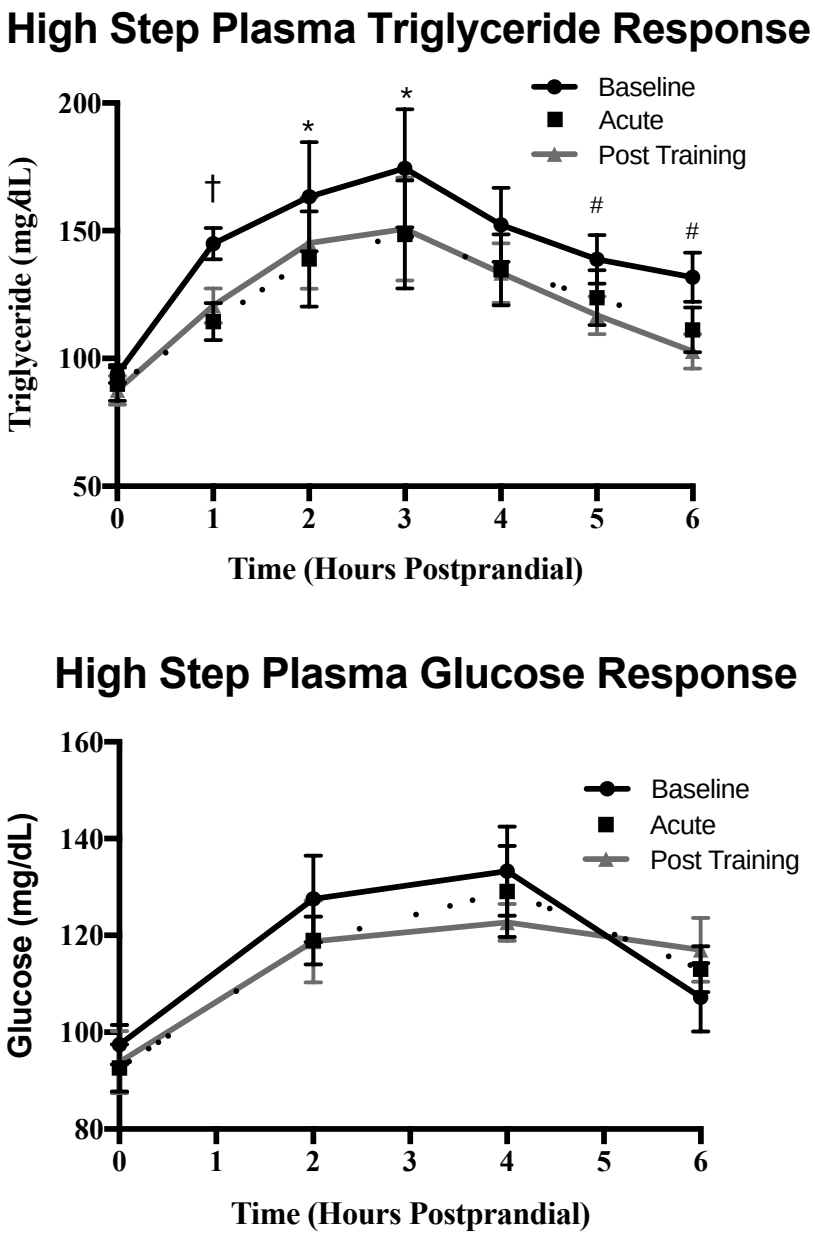


Figure 5. Temporal responses of plasma triglyceride & plasma glucose concentrations for High Step treatment during the HFTT at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). (*) Acute significantly different from Baseline, $p < 0.05$. (†) Acute & Post Training significantly different from Baseline, $p < 0.01$. (#) Post Training significantly different from Baseline, $p<0.05$. Data reported as mean±SE.

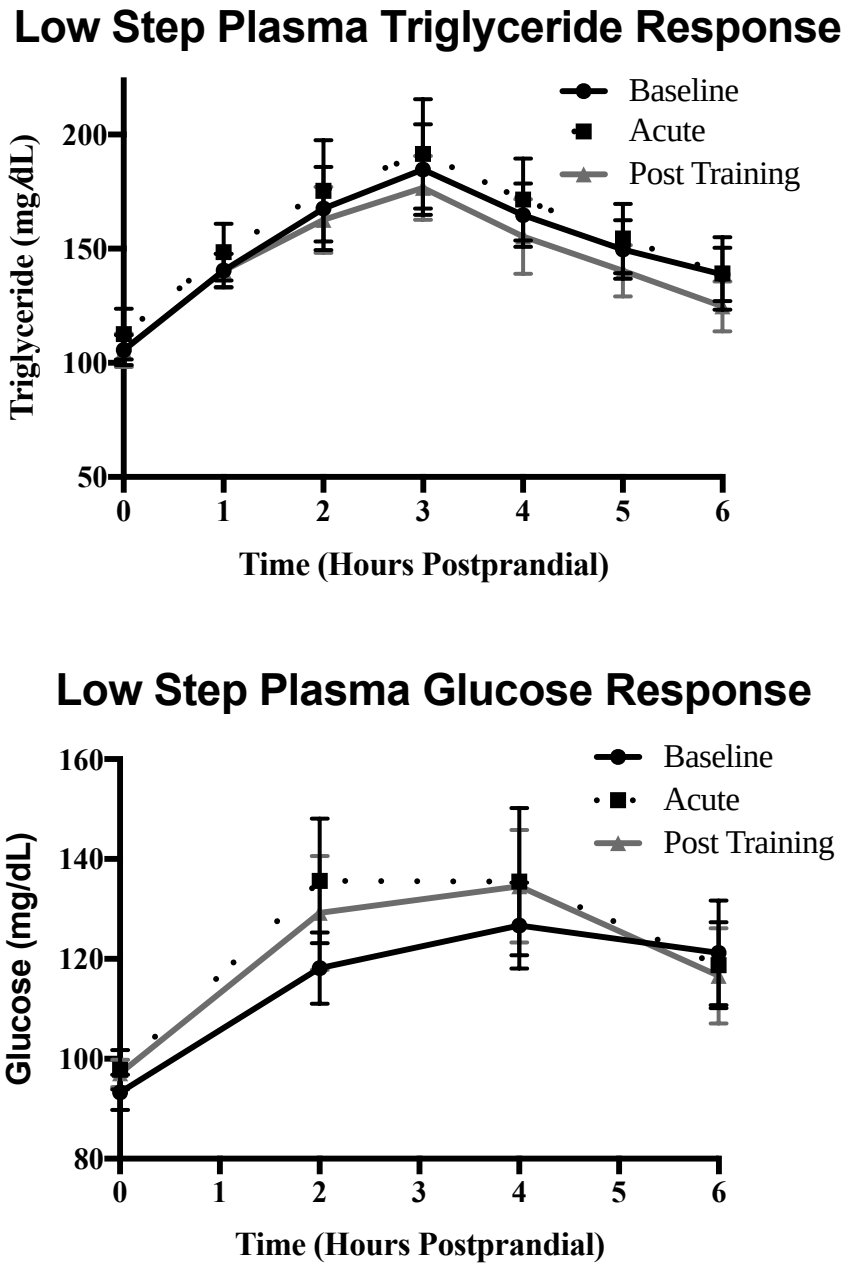


Figure 6. Temporal Responses of plasma triglyceride & glucose concentrations for Low Step treatment during HFTT at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of intervention (Post Training). Data reported as mean±SE.

Physical Characteristics	**High Step (n=8)**	**Low Step (n=8)**
M/F	4/4	4/4
Age (y)	23.4 ± 2.0	23.8 ± 1.4
Height (cm)	166.4 ± 2.8	167.2 ± 3.0
Body Mass (kg)	74.4 ± 5.9	72.6 ± 3.9
BMI (kg/m2)	26.7 ± 1.9	25.9 ± 1.0

Note: Data are reported as Mean±SE

Table 1. Descriptive statistics of the two groups (i.e. High Step and Low Step) at the beginning of the study. All data are reported as mean ± SE.

	High Step		Low Step	
	Pre	*Post*	*Pre*	*Post*
Absolute VO2peak (L/min)	2.51 ± 0.3	2.70 ± 0.2†	2.35 ± 0.2	2.52 ± 0.3*
Relative VO2peak (mL/kg/min)	34.1 ± 3.3	36.9 ± 3.6†	32.2 ± 2.9	34.5 ± 3.3*
Submaximal VO2 (L/min)	1.98 ± 0.7	1.98 ± 0.9	1.87 ± 0.6	1.88 ± 0.2
%VO2peak	78.3 ± 0.8	72.5 ±1.8†	79.9 ± 0.7	75.3 ± 1.3*
Heart Rate (bpm)	181.4 ± 4.7	168.8 ± 4.6†	180.9 ± 5.3	175.8 ± 4.1
HHb (AU)	21.7 ± 4.6	20.1 ± 4.1*	21.1 ± 6.5	22.1 ± 6.3
Blood Lactate Concentration (mmoL)	7.6 ± 0.8	6.7 ± 0.8*	7.2 ± 0.3	7.2 ± 0.4
RPE	15.6 ± 0.8	13.8 ± 0.5	15.8 ± 0.4	14.5 ± 0.6
Workload (W)	135.1 ± 18.7	--	125.6 ± 15.3	--

Note: Data are reported as Mean±SE. (*) Significantly different from Pre, $p<0.05$. (†) Significantly different from Pre, $p<0.01$

Table 2. Physiological responses to maximal and submaximal exercise testing. (*) significantly different from pre-testing within treatment group, $p<0.05$. (†) significantly different from pre-testing within treatment group, $p<0.01$. All data are reported as mean ± SE.

Variables	High			Low		
	Baseline	Acute	Post Training	Baseline	Acute	Post Training
RER	0.83 ± 0.01	0.79 ± 0.01*	0.80 ± 0.01*	0.83± 0.01	0.82 ± 0.01	0.81 ± 0.01
Fat Oxidation (%)	58.5 ± 3.18	70.7 ± 2.32*	67.3 ± 2.10*	57.7 ± 3.89	62.6 ± 2.81	63.9 ± 2.57
Fat Oxidation (kcal/6h)	310.2 ± 18.2	384.1 ± 25.1*	368.9 ± 16.5*	330.4 ± 37.2	351.8 ± 43.4	350.7 ± 29.2
CHO Oxidation (%)	41.5 ± 3.18	29.3 ± 2.32*	32.7 ± 2.10*	42.3 ± 3.89	37.4 ± 2.81	36.1 ± 2.57
CHO Oxidation (kcal/6h)	216.6 ± 19.1	157.8 ± 11.9	181.2 ± 13.7	233.9 ± 22.5	203.5 ± 11.3	201.1 ± 23.1
Total Energy Expenditure (kcal/6h)	526.8 ± 18.7	541.9 ± 26.3	550.1 ± 18.6	564.3 ± 39.6	555.3 ± 45.2	551.8 ± 43.9

Note: Data are reported as Mean ± SE.

Table 3. Overall postprandial substrate oxidation during HFTTs at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). (*) Significantly different from Baseline, ($p<0.05$). Data reported as mean±SE.

Chapter IV: Study #2

DOSE RESPONSE OF PHYSICAL INACTIVITY ON PLASMA TRIGLYCERIDES AFTER A MEAL

Abstract

Background: It has been suggested that there is a linear, inverse dose-response relationship between the daily steps and cardiovascular events. However, it seems if individuals severely reduced the number of steps taken throughout the day the protective effects of exercise may not be realized. The objective of this study was to determine differences in postprandial metabolic responses following acute exercise against a background of differing levels of daily step reduction.

Methods: Ten participants completed three, five-day trials in a randomized, crossover design with differing levels of daily step reduction. Following two days of controlled activity, participants completed two days of Low, Limited, or Normal Activity (2,675, 4,759, or 8,480 steps/day, respectively). Participants also completed a one-hour bout of exercise on the evening of the second day of step reduction. A high fat tolerance test was performed on the following morning. Postprandial responses were compared in each trial.

Results: Daily steps were significantly different in each trial (2,675, 4,759, or 8,480 steps/day, respectively; $p<0.05$) while responses to the acute moderate intensity exercise were similar ($p>0.05$). Following the NORM trial, participants' incremental plasma triglyceride response was lower than LIM by 23% ($p<0.05$) and LOW by 22% ($p<0.05$). Whole body fat oxidation was also significantly increased in NORM compared to the two other trials ($p<0.05$). No significant differences were found between LIM and LOW in any postprandial measure.

Conclusion: In conclusion, two days of daily step reduction in young healthy individuals can impair the ability of acute exercise to attenuate PPL. The finding that the participants

don’t lower PPL or increase fat oxidation in response to exercise when taking ~4700 steps or less, may indicate a reduced responsiveness of skeletal muscle to exercise. This agrees with the newfound phenomenon of ‘exercise resistance’ in individuals whose daily life is characterized by inactivity (e.g. prolonged sitting) and low step count.

Introduction

The cardio-metabolic health benefits of physical activity and exercise such as improved postprandial hypertriglyceridemia and improved glucose tolerance, can be gained acutely from a single bout of exercise and lost with several days of inactivity (80, 82, 83). However, studies in which acute exercise resulted in reduced postprandial hypertriglyceridemia, the participants were accumulating approximately 7,000-8,500 steps on the day before evaluation of postprandial metabolism (99, 183, 184). Although, in a recent study Kim et al. (98), the authors reported that in participants who were sitting for >14 h/day and taking only 1,650 steps/day, a one-hour bout of running at 67% maximal oxygen consumption (VO_{2max}) failed to improve postprandial hypertriglyceridemia the next morning. It seems that physical inactivity (i.e; severely reduced step count) rendered the participants resistant to the normal acute improvements in indices of cardio-metabolic health that are normally derived from a one-hour bout of running. This phenomenon is referred to as 'exercise resistance' (98). A follow-up study in 2019 (1), used additional controls to verify the existence of this phenomenon found a group taking ~3700 steps/day also exhibited exercise resistance. Therefore, it is important to systematically delineate what magnitude of daily step reduction causes impairment of the ability of acute exercise to improve the postprandial plasma triglyceride response.

In modern culture, we have engineered physical activity out of our daily lives. Periods of prolonged inactivity, characterized by mostly sitting, have become routine in the lives of many and routinely coincide with the non-fasting or postprandial state. In the postprandial state, triglyceride levels in the plasma can remain elevated for up to 10

hours, typically peaking 3 - 6 hours after a meal rich in fat (152). The magnitude and duration of this elevation is influenced by prior physical activity (52, 120, 221), diet (174), and genetics (173, 204). As demonstrated in recent epidemiological studies (5, 142), non-fasting plasma triglyceride levels, i.e., *post-prandial lipemia (PPL)*, better predicts cardiovascular events than fasting plasma triglyceride levels and are known to be associated with diseases, including metabolic syndrome, type 2 diabetes, and atherosclerosis. In fact, several recent epidemiological studies, inactivity and/or sitting time has been strongly associated with the risk of obesity, metabolic disorders including type 2 diabetes mellitus and especially with cardiovascular disease and death (16, 198, 218). Surprisingly, some have reported that the risks from prolonged sitting appears "independent" of the volume of exercise being performed (16, 150, 198, 218). This means people who meet the recommended guidelines (AHA or ACSM) for physical activity of 150 min/week of moderate intensity exercise appear to still be at risk for developing cardiovascular disease and all-cause death if they have a lifestyle routinely incorporating prolonged periods of inactive, sedentary behavior (>10-12 h/day).

One of the strongest negative correlations (r=-0.96) in relation to sedentary time is time in light-intensity activity, such as walking (65). This means increasing time spent ambulatory reduces sitting time. Accordingly, manipulation of daily step count has been a popular and potent method of studying the effects of inactivity. Appropriately, increased daily walking has been shown to reduce cardiovascular events (122) while reductions in daily step number for as little as one week have been associated with drastic increases the area under the curve of plasma insulin during an oral glucose tolerance test (OGTT)

(145). This increase showed the potential to grow to nearly 80% greater, if the reductions were maintained for 2 more weeks (145). Daily step reductions have also been linked to decreased VO_{2max}, endothelial dysfunction, decreased insulin sensitivity, decreased lean leg mass and increased abdominal fat (22, 109, 145).

Meanwhile, it is well established that a single bout of moderate exercise lasting 60-90 minutes attenuates PPL regardless of prior lipid levels (221) and training status (66, 120, 151). Many studies (52, 120, 221) have been conducted to investigate the effect of a single bout of moderate intensity exercise on postprandial triglyceride levels in comparison to a control condition. Participants in these studies were asked to refrain from any planned exercise but their ambulatory activity, including walking, was not carefully controlled. Furthermore, very few studies (1, 98) have investigated the collective effect of daily step reduction and moderate exercise. Although recent data (1) present compelling evidence that drastically reducing daily step number may abolish the ability of an acute bout of exercise to attenuate the increase in PPL, some other studies seem to suggest this may not occur with as little as ~7,900 steps/day (183).

Thus, the purpose of this study was to investigate the effect of reductions in daily step number and a single 1h bout of moderate intensity exercise on postprandial concentrations of plasma triglyceride and glucose, as well as fat oxidation. We hypothesized that postprandial responses, following a single bout of 1-hour of running at 65% VO_{2max}, would differ as daily steps increased.

Methods

Ten healthy untrained, recreationally active male (n=7) and female (n=3) participants completed three different trials of differing daily step counts based on previously established cut-points for physical activity (194). Participants were assigned to Low Activity (LOW): 2675 steps/day, Limited Activity (LIM): 4,759, and Normal Activity (NORM): 8480 steps/day in a randomized, crossover design, each occurring over five days with at least a week interval between trials (See Figure 7). Participants were asked to refrain from any planned exercise outside of the experimental design. Participants were given written and verbal description of all the procedures and measurements used in this study, and written informed consent was obtained. The Institutional Review Board of the University of Texas at Austin approved this study (ClinicalTrials.gov Identifier: NCT03697382).

Experimental Design

Each trial consisted of three phases: the first two days served as a control phase (C1 and C2), that allowed for familiarization and control, followed by a 2-day intervention phase consisting Low, Limited, or Normal physical activity consisting of daily step counts of 2,675, 4,759, or 8,480 steps per day, respectively, with 1-h of running on the evening of the second day of each trial at 64% VO_{2max} on a laboratory treadmill. On the morning of Day 3 all participants ingested a high fat shake (i.e.; high fat tolerance tests; HFTT) and the postprandial responses were measured over the subsequent 6h period. Throughout the three trials, participants were instructed to refrain from any

exercise other than that prescribed in the study design. Participants were also asked to keep a consistent sleep/wake cycle during the trials.

Preliminary Testing

One week prior to the initiation of the first trial, participants visited the Human Performance Laboratory (HPL) for a 20-min, 4-stage submaximal test to determine oxygen consumption while jogging at different paces followed by determination of maximal oxygen uptake (VO_{2max}). This served to determine the appropriate treadmill speed to elicit the desired intensity during the 1-h exercise bout. In order to determine VO_{2max} participants performed an incremental treadmill test lasting 8-12 minutes during which the incline was increased 2% every 2 minutes (29). VO_2, VCO_2, and heart rate were monitored throughout the test, and the highest 30 second VO_2 average was recorded for the participant's maximal oxygen consumption. The ACSM criteria for VO_{2max} was used in assessing a successful VO_{2max} test. These criteria are: a plateau in oxygen consumption (less than 150 ml/min increase in VO_2 with increasing work), respiratory exchange ratio (RER) >1.1, maximal heart rate within 10 bpm of predicted maximal heart rate, and a rating of perceived exertion (RPE) of 17 or greater.

Control Phase

Participants were instrumented with an activity monitor worn on their thigh to record step count (activPAL, PAL Technologies, Glasgow, Scotland) and the monitor began recording at 0:00hrs on the first day of the control phase (C1). Participants were asked to remain aware of their step count and to limit steps to 8000 or less to approximate a non-sedentary, low level of physical activity (189). If participants were unable to achieve 8,000 step limit in their first trial, they were the asked to repeat their activity as closely as possible during the control phases of the subsequent trials.

Intervention Phase

During the intervention phase, D1 & D2, participants were asked to remain seated or lying for much of the day to accommodate their assigned level of non-exercise activity (2,675, 4,759, or 8,480 steps/day). On D2 of each trial participants continued to adhere to the assigned step count, but completed a 1-h run at 64.4% VO_{2max} at 18:00h. The steps during this bout of exercise were not included as part of the participants total for D2.

High Fat Tolerance Test (HFTT)

Participants were given a low fat meal the evening prior to high fat tolerance test (HFTT) given that the plasma TG response to a high fat shake (HFS) may be affected by the fat content of a previous meal (42). On the day of the HFTT (D5), participants reported to the laboratory at 07:00 h. Body weight was measured. They then lie on a padded table for 5 minutes before insertion of a catheter into an antecubital vein. A

fasting blood sample was collected 10 min before consumption of a high fat shake (HFS) (mostly melted ice cream and heavy cream; approximately 14.8 kcal/kg (0.8 g, 1.2 g, and 0.2 g/kg BW of carbohydrate, fat, and protein, respectively). Participants were asked to consume the HFS in 5 minutes. Blood samples were collected over the next 6 hours at 0, 2, 3, 4 and 6h post consumption of the HFS. All blood samples collected were transferred to K2EDTA collection tubes (BD), centrifuged at 2,000 g for 15 minutes at 4°C and then stored in -80°C freezer until later analysis. During HFTT, participants were asked to remain seated quietly reading, watching movies, and/or surfing the internet. Participants were allowed to

Postprandial Substrate Oxidation

Postprandial expired gas collection was used for indirectly assessing substrate oxidation. Participants rested in a chair for 10 minutes, followed by expired gas collection through meteorological balloons for 10 minutes at 0, 2, 4, and 6 h. It has been previously demonstrated that inactivity reduces whole body fat oxidation (98).

Energy expenditure and substrate oxidation were calculated from oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER), energy expenditure and substrate oxidation were calculated based on the methods of Lusk (118).

% Energy from carbohydrate (CHO) oxidation = ((RER – 0.707)/0.293) x 100

% Energy from fat oxidation = 100 – % Energy from CHO oxidation

CHO oxidation (kcal/min) = (%CHO oxidation/100) x VO_2) x 5.05kcal/L O_2

Fat oxidation (kcal/min) = ((1-%CHO oxidation/100) x VO_2) x 4.7kcal/L O_2

Energy expenditure (kcal/min) = CHO oxidation + Fat oxidation

Dietary Control

During the course of the study participants were asked to eat to satiety. Participants logged all food and were asked to consume the same foods on the day prior to each HFTT. On the evening prior to the HFTT participants were given a low-fat meal to consume as fat in the previous meal can affect the response to a high-fat test meal (42, 184). Participants were allowed to supplement higher energy expenditure during the LIM and NORM step trials with a small snack but were asked to adhere to a diet standard in macronutrient breakdown (126).

Biochemical Analysis

For plasma triglyceride and glucose concentrations, all blood samples collected were immediately transferred to K_2 EDTA collection tubes (BD Vacutainer, Franklin Lakes, NJ), centrifuged at 3,000 g for 15 minutes at 4◦C. Plasma was then stored in separate aliquots at -80◦C until later analysis. All measurements for each participant were performed in duplicate within the same analysis. Plasma triglyceride and glucose concentrations were measured by a spectrophotometric method using commercially available kits (Pointe Scientific, Inc. Canton, USA). Intraassay coefficients of variation for plasma triglyceride and glucose concentrations were all less than 10%.

Statistical Analysis

Incremental (AUCI) and total area under the curve (AUCT) for plasma triglyceride and glucose were calculated. Once calculated, repeated measures one-way analysis of variance (ANOVA) was used to test for differences. Plasma glucose and triglyceride curves were calculated and analyzed using repeated measures two-way ANOVA (trial x time). Daily step counts were analyzed using repeated measures two-way ANOVA (trial x time). Similarly, respiratory exchange ratio (RER), as well as fat and carbohydrate oxidation, were analyzed using repeated measure two-way ANOVA (trial x time). When interactions were significant, Tukey's honestly significant difference post hoc tests were run. All data were analyzed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA). All data are expressed as mean ± standard error of the mean (SE), unless otherwise noted, the level for statistical significance was set at $p \leq 0.05$.

Results

Participant Characteristics

Participant characteristics are summarized in Table 4. A total of 10 participants were recruited (7 males, 3 females), with all participants completing all three trials. Participants were apparently healthy, young adults (24.0 ± 1.8) that were untrained to recreationally active.

Responses to Maximal and Submaximal Exercise

Responses to maximal and submaximal treadmill running are shown in Table 5. Submaximal exercise bouts elicited a heart rate of 153.9 ± 3.9 bpm and an oxygen

consumption of 2210.2 ± 154 ml/min, which equated to approximately 64.4% of participants VO_{2max}. These responses were indicative of moderate intensity exercise and were not different between trials.

Daily Steps

Daily steps are presented in Table 6. A significant Trial x Time interaction was found daily step count ($p<0.001$). Post hoc analyses revealed no significant differences within or between trials for control days ($p>0.05$). However, daily steps on Day 1 of the intervention were significantly different for all trials (LOW: 2,744±331, LIM: 4,482±318, NORM: 8,431±732). Likewise, during Day 2 of the intervention, excluding exercise steps, daily steps were significantly different for all trials (LOW: 2,605±313, LIM: 5,037±206, NORM: 8,530±420; $p<0.01$ for all comparisons).

Total Plasma Area Under the Curve Responses

Plasma triglyceride concentrations were analyzed at each time point in all trials for all trials and calculated for incremental area under the curve (AUC_I) and total area under the curve (AUC_T) (Figure 8). Analysis of plasma TG AUC_T revealed significant differences with NORM being significantly lower than LOW ($p<0.01$) and a trend toward difference compared to LIM ($p=0.09$). AUC_I in NORM (267.5±39.2 mg/dL*6h) was significantly different from LOW and LIM (342.3±47.8 and 348.6±48.9 mg/dL*6h, respectively, $p<0.05$). No differences were detected between LOW and LIM in AUC_T,

nor AUC_I. Plasma glucose areas under the curve showed no differences ($p>0.05$) between the three trials in AUC_T or AUC_I (Figure 9).

Plasma Triglyceride & Glucose Concentrations

Plasma triglyceride and glucose concentrations were analyzed at each time point in all trials for both treatments. Triglyceride and glucose excursions are shown in Figure 10 for TG, and Figure 11 for glucose. Significant differences existed at multiple time points between trials. At hours 2 and 3, NORM was significantly different from LOW ($p<0.05$). At hour 3, NORM was also significantly different from both LIM ($p<0.05$). NORM was also significantly different from LOW ($p<0.01$) and LIM ($p<0.05$) at hour 4. No differences existed at baseline or hour 6 for triglyceride concentrations ($p>0.05$). Furthermore, no significant differences were found between trials at any time point for the six-hour triglyceride excursion.

Postprandial Substrate Oxidation

Postprandial substrate oxidation was determined using indirect calorimetry (Table 8). Postprandial RER was significantly different in NORM (0.77±0.01) compared to LOW (0.80±0.01, $p<0.05$) with a strong trend toward significant from LIM (0.81± 0.01, $p=0.06$). Similarly, percent fat and carbohydrate oxidation, as well as absolute carbohydrate oxidation (i.e. kcal*6h) were different in NORM compared with LOW ($p<0.05$). Notably, Absolute fat oxidation was significant different in NORM (396.0±27.5 kcal), compared with LOW (318.9±34.5 kcal, $p<0.05$) and LIM (342.4±30.9, $p<0.05$).

Post hoc analysis for bihourly RER measurements (Appendix F) revealed differences for hour 2 between NORM and both LOW and LIM ($p<0.05$), while RER was similar between trials at other measurement time points ($p>0.05$). Similarly, differences in percent fat oxidation and percent carbohydrate oxidation were significant at hour 2. Finally, no difference was found between trials in overall postprandial energy expenditure, or energy expenditure at any single time point between or within trials ($p>0.05$).

Discussion

The purpose of this study was to investigate the effect of daily step reductions on postprandial responses to a high fat meal the morning after an acute bout of moderate-intensity exercise. We hypothesized our key measures, TG AUCI, and fat oxidation would display a curvilinear dose-response relationship with daily steps taken in the two days preceding an acute exercise bout. The primary finding of this study was that when individuals take ~8500 daily steps their postprandial triglyceride responses and whole body fat oxidation during a HFTT, following 1-hour of exercise at 64% VO_{2max}, the night before were significantly improved compared with the same individuals taking 4,759 steps/day or 2,675. In this randomized, cross over experimental design individuals displayed a 23% and 22% reduction in TG AUCI when averaging 8480 steps/day, compared to those same individuals when taking 4,759 or 2,675 steps/day, respectively. The reduction in plasma TG concentration may be due to an increased uptake by tissue and increased oxidation, which was also significantly increased in NORM compared to the other trials.

This is a striking difference as reductions in TG AUCI after similar exercise has been shown to induce TG AUCI reductions on the order of 20-40%, compared to a non-exercise control (46, 99, 183, 221). In other words, when taking 8480 steps/day (i.e.;

NORM), the observed reductions in TG AUCI, compared to both of the LOW and LIM trials, were similar to reductions typically seen compared to a "no exercise" condition. Despite completing identical exercise bouts, the night before commencement of the HFTT, participants seem to have displayed decreased responsiveness to said exercise if they reduced daily steps below ~4,700 steps/day; at least in regards to PPL. Olsen et al (145) found simply reducing steps from ~10,500 to ~1,400 steps/day for two weeks increased TG AUCT by 21% in the absence of exercise.

Recent research has begun to place a particular emphasis on the benefits of increases in daily step counts (107, 111, 189, 191). This is probably due to the ease of translation as a step/day metric is easy to understand and practical, thanks to technology such as wearables that increase the ease of self-monitoring effectively and affordably. Some have recently proposed an inverse dose-response between daily step counts and incidence of cardiovascular disease, type 2 diabetes, and all-cause mortality (107) (111). Lee et al. (111) found rates of mortality progressively declined with increasing daily steps until plateauing at approximately 7,500 steps/day. Contrary to the findings in the present study, the authors reported groups taking as few at 4,363 steps/day displayed reduced mortality compared those taking 2,718 step/day. The difference in findings may be due to the fact that the participants in Lee et al. were substantially older than the population recruited for the current study. Moreover, a recent meta-analysis (107) suggested a 10% reduced risk of cardiovascular events for each 2,000 step increase in daily step number up to 10,000 steps/day. These investigations differ from the findings in this study, as we did not find any improvement in postprandial responses when an individual increased daily walking from ~ 2,700 to ~ 4,750 steps/day. A few important distinctions should be noted and may explain this discrepancy. First, and most obviously, the current investigation focused on responses following moderate exercise which was not employed in the

aforementioned studies. Secondly, the "baseline" step counts in almost all of these investigations, and given as a hypothetical baseline within one (107), exceeded 5,000 steps/day (107, 111, 216) which excludes comparisons below this level of daily walking, such as the 2675 step/day trial presented herein. The current investigation was also conducted over a much shorter time period than the observations. Lastly PPL, while indicative of CVD events, is only a single factor contributing to the development of CVD and should not be considered equivalent or wholly indicative of CVD.

It is possible that at severely reduced step counts (<5,000 steps/day) physiological and metabolic responses differ from those above ~8,000 steps/day. This seems to be buttressed by a growing public health literature that suggest a daily step count at or below 5,000 should be classified as a "sedentary lifestyle index" (189, 191) and should be viewed as a problematic because of the distinct health ramifications seen below this level of activity due to "non-exercise activity deficiency" (64). Though this is a reduction below the level some would consider 'normal', it should not be ignored. In fact estimates from the NHANES study, based on objectively collected accelerometer data, indicate approximately 37% of the US population would fall below this level of daily activity (194).

Our findings are particularly interesting in light of recent findings of a phenomenon termed "exercise resistance" (1, 98). These authors observed individuals taking less than 4,000 daily steps were resistant to the exercise stimulus provided by 1-h of running at ~65% VO_{2max}. In these randomized crossover trials (1, 38, 98) the protective effects of exercise, preventing exaggerated rises in postprandial plasma triglycerides and glucose, were not realized if daily step counts were reduced by imposed sitting of 13 hours or more. It seems by drastically reducing the contractile activity in the study participants, an environment was produced within the muscle that prevented the classic improved response to the exercise stimulus. It has been postulated in a recent meta-analysis by Ekelund et al (39)

that individuals experiencing high levels of daily inactivity are at an increased risk of mortality even when participating in similar levels of daily activity (e.g. MET hours). Taken together it seems that reduced contractile activity causes a condition in which current exercise recommendations may not be enough to derive protective benefits. Thus a higher minimum level of recommended physical activity may be needed for populations regularly experiencing prolonged inactivity.

Muscle lipoprotein lipase (LPL) is the rate limiting enzyme for clearance of plasma triglycerides (202). Therefore, decreased LPL activity is a rational candidate for explaining the increased PPL found in this study with LOW and LIM. Although not measured directly in the present investigation, low levels of contractile activity have been observed to drastically reduce the activity in LPL in muscle. In an animal model, hind-limb immobilization has been shown to have sizeable reductions (i.e. 90% decrease) in LPL activity and developed rapidly (>60% reduction in <12h) (15). However, it seems the downregulation is post-transcriptional. Even in the tissue with more than 90% reduction in LPL activity, LPL mRNA was similar to baseline (15). Additionally, the data from this study (15) indicate LPL mRNA is not increased with walking or levels of contractile activity associated with maximal increases in LPL activity. It seems LPL activity may be down regulated by GPIHBP1 protein endocytosis induced by some co-factor produced during periods of prolonged inactivity (12, 94). However, this hypothesis was not tested in the present study and needs additional investigation to fully elucidate a mechanism.

It is plausible that this study would have a greater impact if it were conducted in an older population. This is due to the increased magnitude and duration of the postprandial triglyceride elevation in older populations, compared with their younger counterparts (11, 62). Postprandial TG concentrations have been observed to peak 2-3 hrs earlier in young people while aged participants showed increased rates of chylomicron accumulation

peaking at concentrations nearly four-fold greater (141). The durations of these elevations above baseline mirrored these findings, returning to baseline after 6 hours in the young compared to 24 hours in an older population (11). However, it has been postulated that this increased PPL is due to the decreases in non-exercise activity associated with aging (62). Moreover, technological advances have drastically decreased occupational physical activity such that activity in much of the workforce can approximate that of sedentary, elderly individuals (26). This is not obviated completely even in health-conscious individuals. For example, 'workday' sedentary time in 208 marathon and half-marathon participants was observed to be similar to those seen in the elderly in assisted living communities (90, 206). Thus, this work addresses a population that may still be at significant health risk.

In conclusion, to the best of our knowledge the current investigation is the first to indicate that 2 days of step reduction can decrease an individual's responsiveness to an acute aerobic exercise bout in terms of stimulating improved PPL and fat oxidation. When participants took 8,480 daily steps and performed a 1h bout of exercise, their responses in TG excursions and AUCs were significantly lower than following the same exercise with step counts at 2,675 and 4,759 steps/day. When viewed from the perspective of previous literature, the reduction in TG AUCI when participants took 8,480 steps, compared to either of the lower daily step counts in this study, were similar to expected reductions that have been observed to occur in a non-exercise control (46). This may support the exercise resistance phenomenon recently coined by Kim et al (98), in which individuals who spend the majority of their day sitting and take relatively few steps are unable to reap the benefits generally associated with acute aerobic exercise (39, 124). Based on these data, from the current investigation and others, it seems that reducing daily steps may cause development of a condition in which the inactive muscle demonstrates blunted responses to normal,

healthy stimuli. Recent observations support this contention in that not only PPL is impaired but reduced myofibrillar protein synthesis can be seen in elderly (22) and young healthy individuals (166) in response to step reductions to ~1400 steps/day of two weeks or less. These findings coupled with previous work on exercise resistance (1, 38, 98) emphasize the necessity of maintaining a sufficient amount of physical activity (i.e.; >8,500 Steps/day) to ensure healthy PPL responses, even in participants exercising for 1h at 64% VO2max.

Tables and Figures

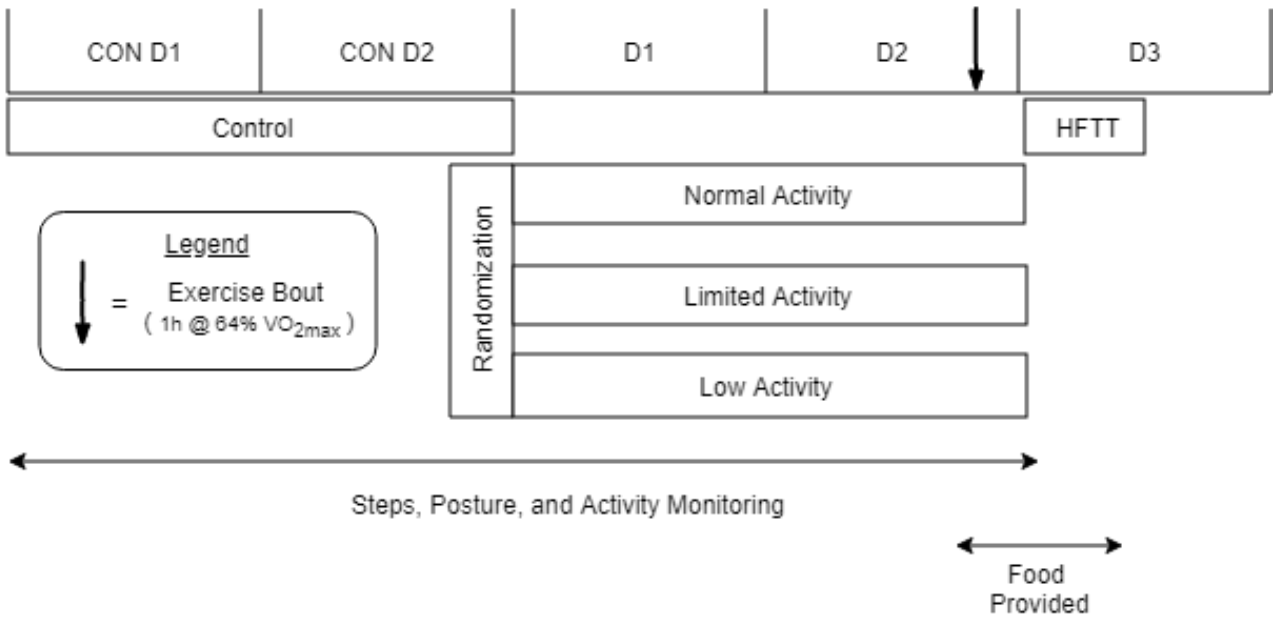


Figure 7. Study Design. Participants completed a five-day randomized, crossover experimental design with differing levels of daily step reduction (i.e. Low- 2,675, Limited- 4,759, & Normal Activity-8,480 Steps/Day). Participants completed two control days with activity monitoring before the initiation the two-day step reduction (D1 & D2). Participants also completed an hour of treadmill running on the night of D2 followed by HFTT on the morning of D3.

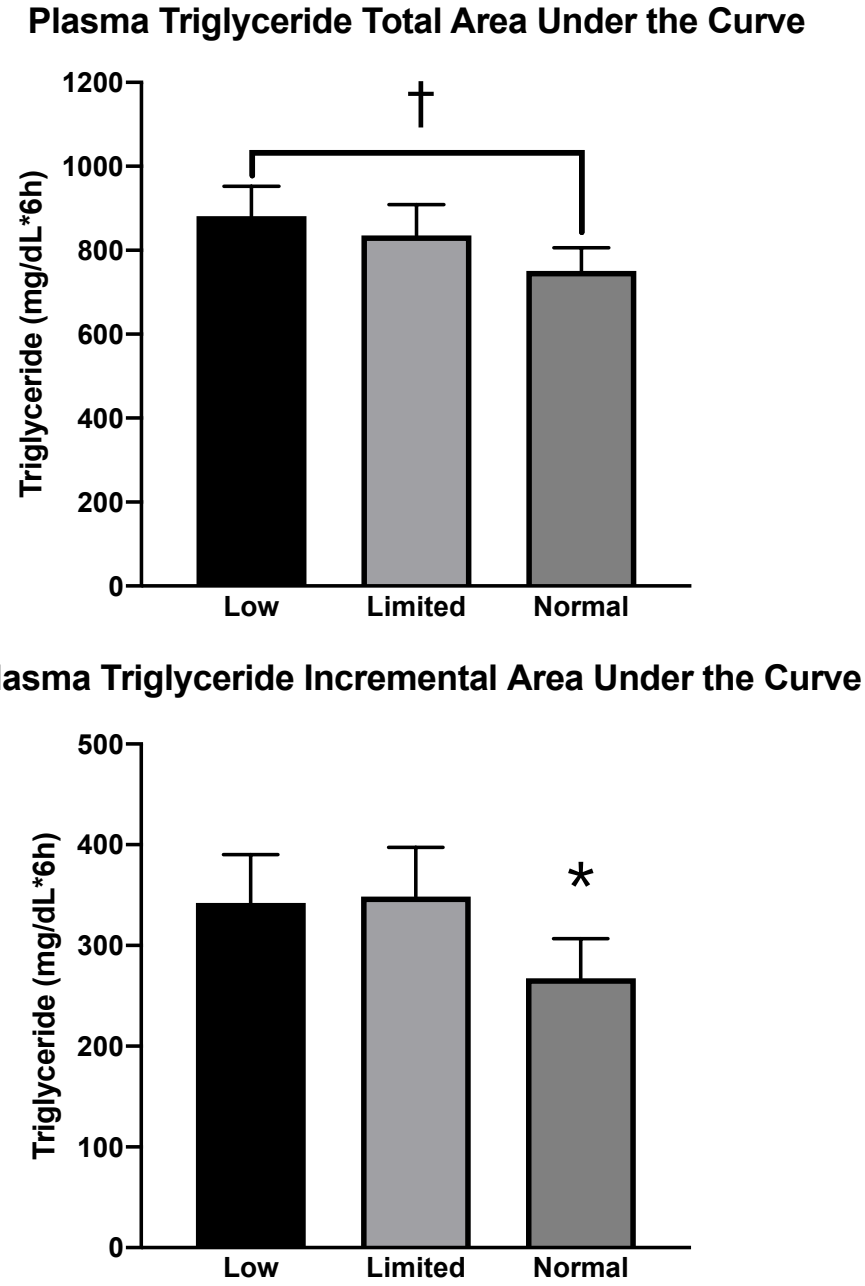


Figure 8. Total and Incremental areas under the curve of plasma triglyceride concentrations during HFTT for each trial. (*) significantly different from Low & Limited step group, $p<0.05$. (†) significantly different from Low step group, $p<0.01$. Data reported as mean±SE.

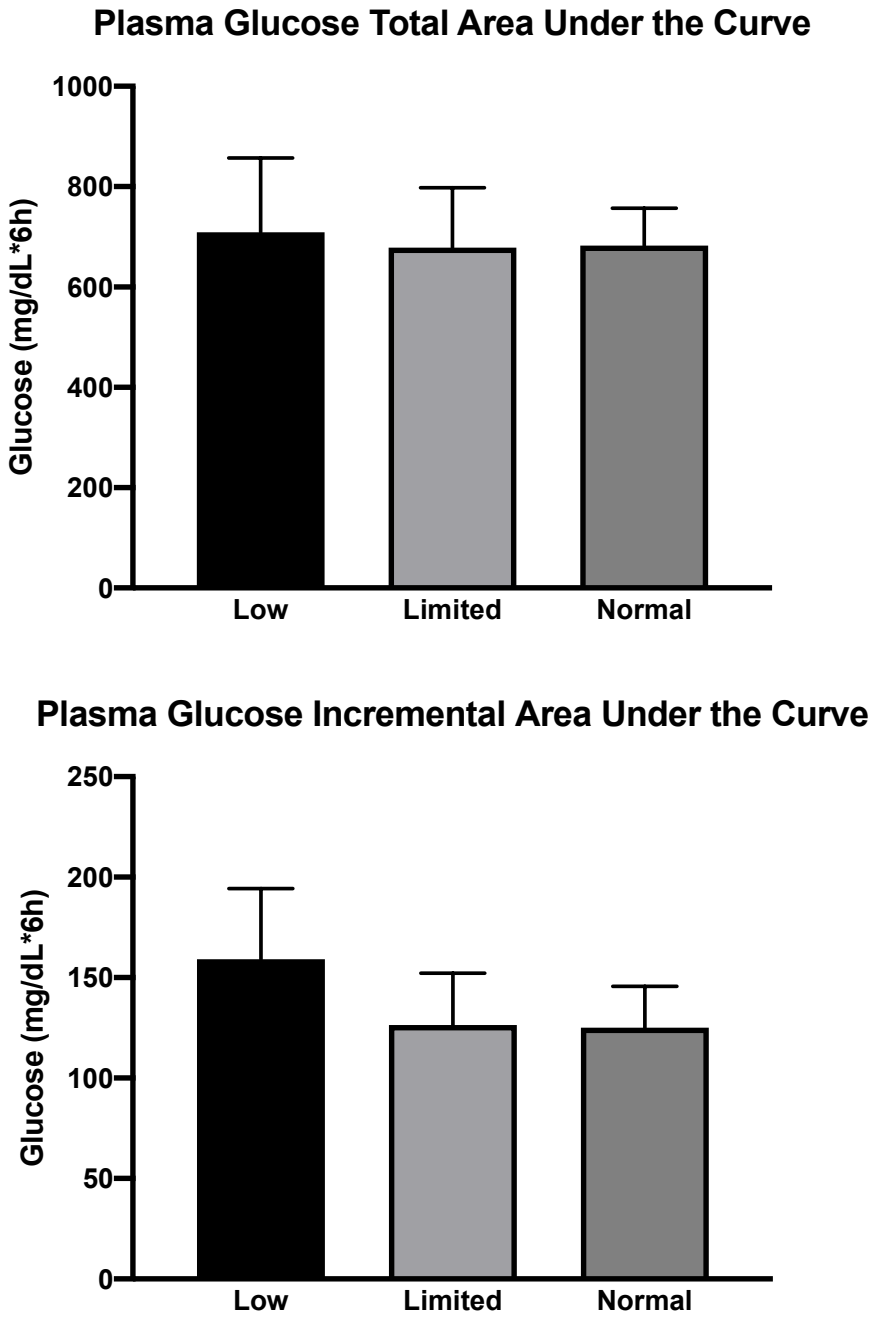


Figure 9. Total and Incremental areas under the curve of plasma glucose concentrations during HFTT for each trial. Data reported as mean±SE.

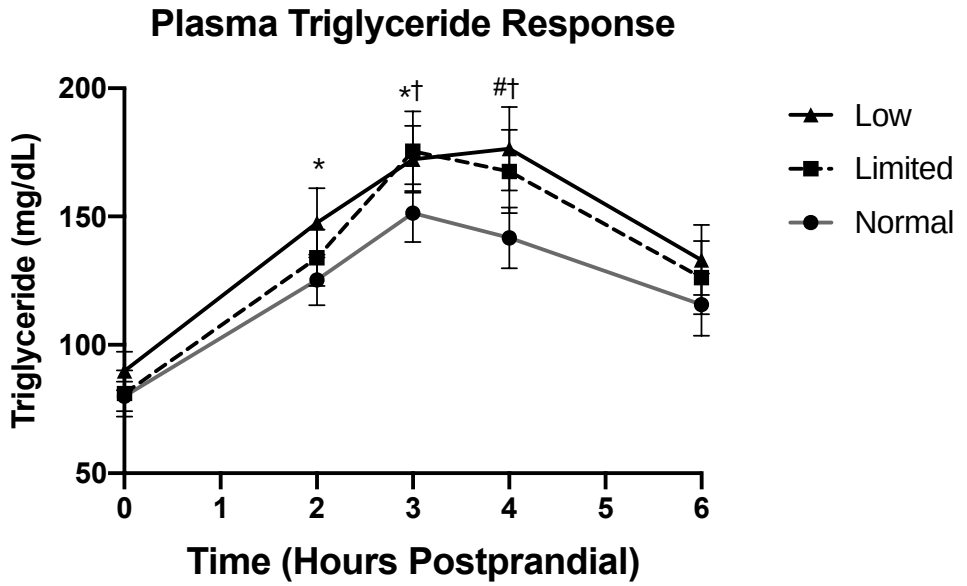


Figure 10. Temporal Responses of plasma triglyceride concentrations for each trial during HFTT. (*) Normal significantly different from Low, $p<0.05$. (†) Normal significantly different from Limited, $p<0.05$. (#) Normal significantly different from Low, $p<0.01$. Data reported as mean±SE.

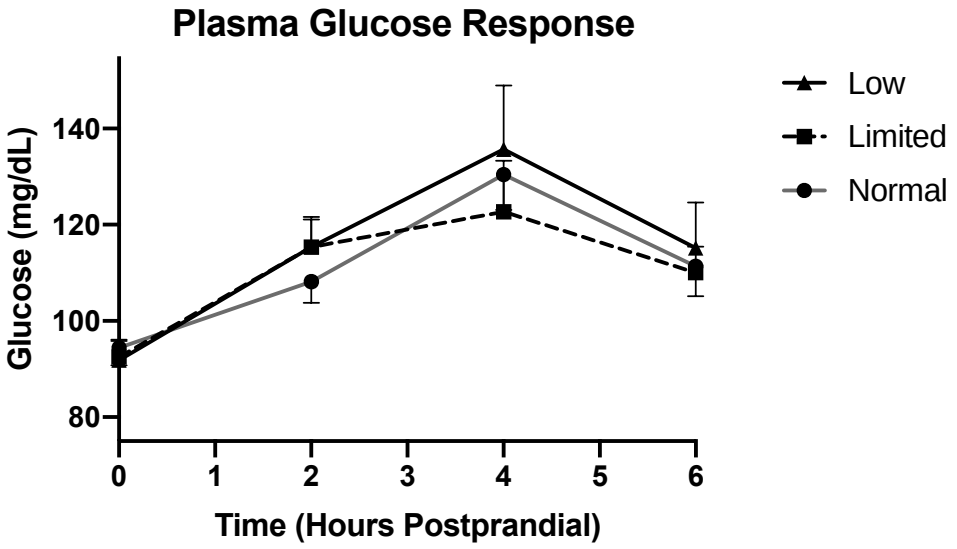


Figure 11. Temporal Responses of plasma glucose concentrations for each trial during HFTT. Data reported as mean±SE.

Physical Characteristics	mean ± SE
Age (y)	23.4 ± 5
Height (cm)	166.4 ± 7.9
Body Mass (kg)	74.4 ± 16.9
BMI (kg·m^{-2})	26.7 ± 5.2

Note: Data are presented as M±SE

Table 4. Descriptive statistics for participants at the beginning of the study. All data reported as mean ± SE.

Exercise Responses	mean ± SE
Maximal Oxygen Consumption	
VO_{2max} Absolute (ml/min)	3,405 ± 242
VO_{2max} Relative (ml/kg/min)	42.7 ± 1.4
Submaximal Exercise During 1-h Run	
Heart Rate (bpm)	154 ± 4
Rating of Perceived Exertion	11.4 ± 1.1
VO_2 (ml/min)	2210 ± 154
% VO_{2max}	64.4 ± 0.4
Treadmill Speed (mph)	4.8 ± 0.2

Note: Data are presented as M±SE

Table 5. Responses to maximal exercise and the 1-h bout of submaximal exercise. All data reported as mean ± SE.

	Trial	Day of Trial			
		C1	C2	D1	D2
Daily Steps	Low	10198 ± 1476	11015 ± 1159	2744 ± 331	2605 ± 313
	Limited	9808 ± 1207	10568 ±1112	4482 ± 318*	5037 ± 206**
	Normal	11056 ± 1324	10732 ± 936	8431 ± 732†	8530 ± 420†

Note: Data are presented as M±SE. (*) Significantly different from Low, p<0.05. (**) Significantly different from Low, p<0.01. (†) Significantly different from both Low & Limited, p<0.01

Table 6. Average daily steps measured via activPal activity monitor, attached on the participant's anterior thigh throughout each trial. Average daily step counts for each trial are presented for Control (C1 & C2) and Intervention Phases (D1 & D2). (*) significantly different from Low, p<0.05. (**) significantly different from Low, p<0.01. (†) significantly different from Low & Limited step trial, p<0.05.

Trial	Postprandial Time (Hours)				
	Baseline	H2	H3	H4	H6
Triglyceride Concentration (mg/dl)					
Low	89.8 ± 7.5	147.5 ± 13.5	172.4 ± 13.0	176.5 ± 16.2	133.0 ± 13.6
Limited	81.1 ± 9.1*	134.0 ± 11.1	175.4 ± 15.5	167.6 ± 16.3	126.2 ± 14.2
Normal	80.0 ± 5.8	125.3 ± 9.8*	151.3 ± 11.3*†	141.6 ± 11.9*†	115.7 ± 12.2
Glucose Concentration (mg/dl)					
Low	91.8 ± 4.3	116.4 ± 6.7	---	139.7 ± 16.8	116.3 ± 10.4
Limited	92.5 ± 3.4	115.4 ± 5.7	---	122.7 ± 10.6	110.1 ± 5.4
Normal	94.5 ± 3.7	108.2 ± 4.4	---	130.4 ± 7.3	111.4 ± 6.2

Table 7. Hourly responses (e.g.; H2, H3, etc.) of plasma triglyceride and plasma glucose concentrations during HFTT for each trial. (*) Significantly different from Low, $p<0.05$. (†) Significantly different from Limited, $p<0.05$. Data reported as mean±SE.

Variables	Treatment Group		
	Low	Limited	Normal
RER	0.81 ± 0.01	0.80 ± 0.01	0.77 ± 0.01*
Fat Oxidation (%)	66.1 ± 4.87	69.6 ± 3.93	80.4 ± 2.65*
Fat Oxidation (kcal/6h)	318.9 ± 34.5	342.4 ± 30.9	396.0 ± 27.5*
Carbohydrate Oxidation (%)	33.9 ± 4.87	30.4 ± 3.93	19.6 ± 2.65*
Carbohydrate Oxidation (kcal/6h)	164.0 ± 25.3	149.0 ± 24.1	97.8 ± 12.8*
Total Energy Expenditure (kcal/6h)	482.9 ± 32.6	491.4 ± 31.2	493.8 ± 27.0

Table 8. Overall postprandial substrate oxidation during HFTT for each trial. (*) significantly different from Low & Limited, $p<0.05$. Data reported as mean±SE.

Chapter VI: General Summary

These studies were conducted in order to determine: 1) if the background level of daily physical inactivity impairs postprandial lipemia (PPL) and cardiovascular adaptations to short term training and 2) the effect of altering daily step counts for two days on the ability of a 1-h bout of moderate-intensity exercise to reduce PPL.

In Study 1, it was shown that inducing physical inactivity by reducing daily step count to 4,767 steps over 11 days, in conjunction with vigorous-intensity exercise training, resulted in an inability to incur the classic PPL-lowering effect of acute exercise as well as short term training. Furthermore, classic adaptations, such as decreases in heart rate and blood lactate concentration and increases in muscle oxygenation and fat oxidation when exercising at an absolute workrate, typically seen with exercise training and displayed in the High Step group ($p<0.05$), were not significant in the Low Step group. This is consistent with the findings of Kim et al. (98) that first coined the term 'exercise resistance', but also extends this phenomenon to an impairment of short-term training adaptations as well as measures of postprandial metabolism. These data indicate that, even in individuals who participate in the early stages of regular exercise training, background inactivity results in elevated PPL and impaired cardiometabolic adaptations to short term training.

In Study 2, it was demonstrated that reducing daily steps to 4,759 or below is associated with a decreased ability of acute exercise to lower PPL, compared to a trial taking 8,480 steps/day. Responses to a high fat meal on the morning following a 1-h run at 64% of VO_{2max} were similar in groups taking 2,675 (LOW) and 4,759 steps/day (LIM). However, following an identical exercise bout, participants taking 8,480 steps/day (Normal) significantly reduced their plasma triglyceride incremental area under the curve compared to LOW and LIM trials ($p<0.05$). This indicates that the development of the

aforementioned ‘exercise resistance’ occurs when steps/day drop below approximately 5,000. These findings suggest that some beneficial effects of acute exercise can be diminished if individuals experience regular periods of inactivity leading to reduced daily steps (i.e. below approximately 5,000 steps/day).

Taken together, these studies suggest deleterious effects of inactivity on an individual’s ability to properly respond in terms of cardiometabolic adaptations (i.e; PPL, HR, lactic acid, fat oxidation and muscle oxygenation) to an exercise stimulus provided by 1-h of running or 9 days of chronic training. The Low Step treatment group in study 1 took approximately 5,000 steps/day which is similar to the limited step trial (LIM) in study 2. This is useful in comparing the effects of reduced step counts in both studies. Taking ~5,000 steps/day seems to have rendered the exercise training ineffective in lowering PPL in study 1 in that postprandial responses following training were not significantly different from baseline responses. Additionally, data from study 2 shows acute exercise while taking ~5,000 steps/day or less (i.e. LIM & LOW trials) is less effective at lowering PPL compared to taking ~8,400 steps/day. Jointly the findings in this dissertation suggest that < 5,000 steps/day is insufficient to realize the protective effects of acute or short term exercise training, at least in terms of PPL and fat oxidation (Table 9). The exercise resistance phenomenon first postulated by Kim (1) seems to manifest itself when taking less than 5,000 steps/day, regardless if the exercise is of moderate or vigorous intensity and persists even with regular, short-term training. It should be realized however, that although taking less than 5,000 steps/day appear ineffective in preventing exercise resistance, and taking approximately 8,500 steps/day is effective, it is still unclear how activity levels between these step counts affects the development of this phenomenon.

These findings suggest that reductions in daily step counts are associated with impaired cardio-metabolic responses (i.e.; postprandial lipemia and fat oxidation) to both

acute and short term exercise training. These studies have some noteworthy strengths in that they add pertinent information to the literature especially surrounding the newfound "exercise resistance" phenomenon. To the authors knowledge this is the first study to consider the interplay of physical inactivity and short-term training. Moreover, the second study provides important information on hoe to characterize the onset of this new phenomenon. Lastly, these findings suggest that even young, healthy individuals are susceptible to negative cardio-metabolic effects of physical inactivity that cannot be easily overcome with exercise.

	Trial		
Study 1 (Training)	HIGH STEP	LOW STEP	
Steps per Day	16,048	4,767*	
Study 2 (Acute)	NORMAL	LIMITED	LOW
Steps per Day	8,431	4,759 *	2,744*

Table 9. Summary of findings (*) signifies impaired metabolism

Chapter VII: Review of Literature

Introduction

The perils of physical inactivity have been generally appreciated for several millennia. Susruta, an Indian physician in the 7th century BC, may have been the first physician to prescribe daily activity. He believed daily physical activity was necessary to ward-off disease, even stating "diseases fly from the presence" of such individuals participating in habitual physical activity (177). Not long after in the 5th century BC, Hippocrates is quoted as saying "walking is man's best medicine" and, "all parts of the body… if they are unused and left idle, become liable to disease…" (106). Moreover, the effects of physical inactivity have been observed and studied systematically since the 1950's. The seminal work by Morris et al. (133) found an increased rate of coronary artery disease in London bus drivers compared with their more active counterparts on the busses, the conductors. These findings suggest that individuals who are chronically inactive can suffer serious detriments to health as a result. Since then, numerous studies have been conducted to evaluate the deleterious effects of physical inactivity. However, even with the recent interest and the emergence of the field of inactivity physiology (63), it seems we have only scratched the surface in understanding the harmful effects of inactivity and its increasing prevalence in many modern cultures.

In modern culture we are continually engineering activity out of our daily lives. As such, periods of prolonged inactivity have become routine in the lives of many. This is especially prevalent in well-developed countries in which technological advances allow

for increased automation and vehicular transportation (217). The populations of such societies also rarely go more than 8 hours without eating. Consequently, these individuals spend most of their time in a non-fasting or postprandial state. As a consequence of an increasingly sedentary lifestyle, periods of prolonged sitting and the postprandial state routinely coincide resulting in chronically elevated levels of plasma triglyceride and glucose. The consequences of prolonged sitting have begun to be recognized by some countries who have already made a conscious effort to advise against prolonged bouts of sitting (217). In the postprandial state, triglyceride levels in the plasma can remain elevated for up to 10 hours, typically peaking 3 - 6 hours after a meal rich in fat (152). The magnitude and duration of this elevation is influenced by prior physical activity (52, 120, 221), diet (174), and genetics (173, 204). As demonstrated in recent epidemiological studies (5, 142), non-fasting plasma triglyceride levels, i.e., post-prandial lipemia (PPL), better predicts cardiovascular events than fasting plasma triglyceride levels and are known to be associated with diseases, including metabolic syndrome, type 2 diabetes, and atherosclerosis.

In several other recent epidemiological studies, sitting time has been strongly linked with the risk of obesity, metabolic disorders including type 2 diabetes mellitus and especially with cardiovascular disease and death (16, 198). Exercise as an intervention has been studied with promising results (76, 120, 222). However, recent epidemiological studies have reported that the risks from prolonged sitting appears “independent” of the volume of exercise being performed (16, 150, 198). This means individuals who meet the recommended guidelines (AHA or ACSM) for physical activity of 150 min/week of

moderate intensity exercise appear to still be at risk for developing cardiovascular disease if they have a lifestyle routinely incorporating prolonged periods of sitting (>10-12 h/day) (39). In 2016, the term 'exercise resistance' was first introduced to describe a phenomenon in which individuals who experience prolonged periods of inactivity seem unable to realize some of classic metabolic benefits associated with an acute bout of aerobic exercise (98).

Postprandial Metabolism and Health

In order to best analyze the effects of physical inactivity on health, it is necessary to establish a measure that can serve as a proxy for cardiometabolic health and is sensitive to the changes associated with inactivity. The method that seems to be the most promising is the measurement of blood lipids, in both the fasted and non-fasted state. Much is understood about the dangers linked to elevated fasting plasma triglyceride levels (79, 121). However, recent epidemiological and scientific evidence suggests postprandial lipemia (PPL) is a stronger indicator of CVD risk than is fasting plasma triglyceride level (5, 87, 88, 142).

Chronic dyslipidemia and the oft-resultant atherosclerosis are two of the principal contributors to CVD (87, 207, 223, 224). Within first-world countries, where food is plentiful, a significant amount of time is spent in the postprandial state, leading to longer periods of elevated triglycerides. These increased PPL levels are associated with reduced high-density lipoprotein production and increased low-density lipoprotein cholesterol

production (44), impaired endothelial function (199), and increased atherosclerotic plaque formation (223).

There are two major sources of circulating triglycerides. These can be produced from an endogenous pathway within the liver (e.g. lipogenesis) or can be consumed through exogenous dietary sources (73). Dietary consumption can lead to two different ultimate destinations of exogenous lipids, depending upon their type. Short-to-medium chain fatty acids are transported primarily to the liver, then successively to their final destination, often being skeletal muscle, undergoing beta oxidation to aid in successfully supplying the energy needs of the tissue. However, triglycerides composed of long chain fatty acids, are carried predominantly via chylomicrons and/or very low-density lipoproteins (VLDL), and transported to adipose and muscle tissues (144). The main transporter of endogenous lipid production are VLDLs. Conversely, for ingested triglycerides, chylomicrons are the primary vehicle (56, 140). Chylomicrons are formed in the endoplasmic reticulum of small intestine enterocytes and are secreted into the lymphatic system. Subsequently chylomicrons travel through the lyphatic system and enter systemic circulation via vena cava (86). In the postprandial state, the formation of chylomicrons may compete with lipoproteins to interact with lipoprotein lipase (LPL), an enzyme located on the luminal side of vascular endothelial cells in adipose, skeletal muscle, and myocardial tissue (18, 32, 201). LPL hydrolyzes the triglycerides from both VLDL and chylomicron sources. However, after a meal as the chylomicrons concentration increases, VLDL production continues as it is modified by liver concentrations of FFAs. The result is a level of circulating triglycerides and cholesterols

that is elevated significantly (223). As chylomicrons and VLDLs both utilize LPL as an uptake mechanism, postprandial saturation of LPL can occur as the two molecules compete for binding sites (32, 201). Therefore, this increase in circulating cholesterol/triglycerides and concomitant saturation of LPL allows for the opportunity for chylomicron and VLDL byproducts to build up in the subendothelial space. During instances where dietary consumption leads to an increase in PPL. Furthermore, residual fatty acids from chylomicron hydrolysis may be re-esterified in the liver as VLDL and eventually used to synthesize various byproducts, including low-density lipoproteins (LDL) (55, 87). It is this production and accumulation of LDL, and other VLDL remnants which is the genesis of atherosclerosis (59, 223, 224).

Atherosclerosis has numerous sources that contribute to its development including endothelial dysfunction (3, 33) and abnormal blood lipids (134, 142, 223). The most common explanation for the onset of atherosclerotic formation begins with an increased accumulation of lipoprotein behind the endothelial wall of the vasculature (117, 138, 209). Here these lipoproteins undergo modification through oxidation, lipolysis, proteolysis, and aggregation. Eventually fostering the formation of foam cells via macrophage infiltration/conversion and to inflammation of the surrounding tissue (117, 172). The resulting damage is eventually repaired but leaves behind underlying tissue which may begin to form a necrotic core and exterior calcification. This tissue is then vulnerable to later injury which may cause release of the interior contents of the lesion and can lead to thrombosis (117, 171). This thrombosis is a likely cause of cardiovascular events such as stroke or myocardial infarction.

Prevalence of Inactivity in Modern Culture

The magnitude and consequences of physical inactivity in modern times have led to it being appropriately termed a global pandemic (105). In order to understand the prevalence of inactivity in a modern lifestyle, it is important first to define the terms by which one would be considered inactive. In 1995 the Centers for Disease Control and Prevention (CDC) and American College of Sports Medicine (ACSM) published minimum recommendations prescribing 30 minutes of moderate intensity exercise on most, preferably all, days of the week (149). These guidelines were recently updated with additional support for beneficial effects of physical activity and the removal of a 10-minute minimum duration for activity (157). This level of physical activity equates to about two miles of brisk walking, accumulated throughout the waking hours of an individual's day. Physical inactivity is generally defined as failing to meet these requirements. Specifically, failure to achieve 150 minutes of weekly moderate to vigorous physical activity (MVPA), 75 min of vigorous physical activity, or a combined equivalent achieving 600 metabolic equivalent (MET)-minutes per week (208, 213, 214). Based on accelerometer data from the National Health and Nutrition Examination Survey, the prevalence of meeting these guidelines could be as low as 5% in the United States (182). Other more hopeful, yet still concerning estimates put this number at about 31% meeting guidelines (60).

It is necessary to understand why this number is so low. Much of the increase in inactivity can be linked to modernization of the culture and the technology associated with said modernization. As societies continue to advance, technology must improve in order that the time spent in menial tasks can be better invested in more meaningful pursuits which spur further progress. However, it seems rather than current technology providing time savings that can be reinvested in other activities, it is instead being replaced by inactive behaviors causing a dramatic rise in daily inactivity.

For example, estimates of daily step numbers indicate the introduction of powered machinery has causes a decrease of 50-70% of daily activity (19, 20, 143). Even in modern times populations, such as some Amish communities, who limit or completely abstain from the use of many modern technological conveniences take four times more daily steps as those who do not (9, 10). This is bolstered by observations like the 2017 American Time Use Survey which revealed just over 5% of leisure time is spent in "exercise, sports, and recreation" (196). It seems that the lives of many are dominated by sedentary actives such as prolonged sitting. Some have suggested it is possible, if not likely, that 95-97% of an individual's waking hours could be spent in sedentary activities (72, 205). Sedentary activities are defined as activities that involve energy expenditure at the level of 1.0-1.5 metabolic equivalent units (METs)(148). More tangibly, sedentary behavior includes activities that do not increase energy expenditure markedly above the resting level. Such as sleeping, sitting, lying down, and watching television. While exercise is prescribed broadly, a 30-minute daily session still allows for upwards of 16 hours of inactivity. Further, evidence suggests that there may be no difference in sitting

time between those who achieve recommended levels of physical activity and those who do not (30).

Engagement in light-intensity activity may be the best way to reduce sedentary behaviors. One of the strongest negative correlations (r=-0.98)(71) in relation to sedentary time is time in light physical activity, such as walking, which involves energy expenditure at the level of 1.6-2.9 METs. (65, 139, 148). While the energy expenditure may only be slightly above that of sitting, these activities are the predominant determinant of overall daily energy expenditure - even in those who exercise regularly (34). Unfortunately, modern cultures fall short on this score as well. Tudor-Locke et al. (194) provides data suggesting 17% of US adults take fewer than 2,500 steps per day. Further about 37% take fewer than 5,000 steps, with anything under this level being considered by most to be indicative of a very inactive or sedentary lifestyle (163, 191, 194). A decrease of 2,500 steps/day is associated with increases in sitting time on the order of 37-45 mins/day (191) or as much as 75 minutes (128).

Another area that has seen a dramatic rise in inactivity is occupational time. (26, 101). Nearly half of occupations in the 1960s could be characterized as requiring moderate activity. That number had dropped to less than 20% by 2008 and this trend is expected to continue (26). These authors concluded that the decrease from occupational energy expenditure over 4 decades could almost entirely explain the increase in weight seen in the NHANES study over the same period (26). This trend effects even those who are most active. Data from one study (206), more clearly illustrates this alarming point. In this study, marathon and half marathon participants who, by the nature of their

recreational choices, are presumably very active were found to have daily sitting times roughly equivalent to those found in elderly community-dwelling populations during the work week (90, 206).

Deleterious Effects of Inactivity

The prevalence of inactivity is extremely concerning, especially given the effects on the health of millions. While the health hazards do not command the same attention from governments and health organizations, the deleterious effects of inactivity are similar to, if not greater than, those seen with smoking and obesity (112). The impact of these on an individual health can be more hazardous but, the prevalence of inactivity is much higher than either smoking or obesity, leading to an effect on the population that is no doubt more severe. In 2009, physical inactivity was officially recognized as the fourth independent risk factor for non-communicable diseases and accounted for more than 5 million preventable deaths per year (112, 213).

It is estimated that 20% of all CVD and 10% of strokes occur due to physical inactivity (35) and over 30% of ischemic heart diseases (105). According to data published in *The Lancet*, inactivity is a causal factor in 9% of premature mortality (112). Further, a reduction in the prevalence of inactivity by just 25% worldwide would prevent over 1.3 million deaths each year (112). Well-reasoned estimates contend that if every person in the United States were to meet physical activity guidelines nationwide, life expectancy would increase by at least 0.68 years on global life expectancy (112). This

number may seem low, as discussed by the authors, but when viewed in the proper light it is actually substantial. That is, those who are already sufficiently active would see no increase in life expectancy, therefore inactive individuals under this estimate could see an increase of four or more years in life expectancy.

One of the starkest examples illustrating the potency of inactivity to cause deleterious health outcomes is the classic Dallas Bed Rest Study. In this study, trained participants underwent 20 days of bed rest. This intervention induced reductions of over 25% in VO_{2max}, cardiac output, and stroke volume and an 11% decline in total heart volume (162). In a follow-up study, McGuire et al. found that these decrements were greater than the decline in these variable resulting from 30 years of aging (125). Given the inverse relationship between cardiorespiratory fitness and all-cause mortality (103) it is clear that inactivity, at least with respect to this level of inactivity, is particularly unhealthy.

It is important to understand why these concerns are especially pertinent to first world countries like the US in which heart disease is the number one cause of death (102). CVD and premature mortality seem to be strongly linked and show a direct dose response relationship to physical inactivity (39, 104). Therefore, several other models of inactivity have been employed in studying the deleterious effects that lead to these health outcomes. Dunstan et al (2004) observed that, when comparing TV time, those who spent the most time watching television were more likely to have diabetes. Additionally, when comparing those with the most TV time to group with the least reported time,

women were more likely to have compromised glucose tolerance. These results held even after adjusting for total time in physical activity as well as other covariates (37). Another analysis of prospective data (84), found a significant trend for increasing obesity and Type II diabetes risk across categories of increasing TV viewing time. Again, this trend remained significant after adjusting for covariates including a measure of exercise participation. Katzmarzyk, et al. (93) provided data from a prospective study of physical activity, sitting time and mortality. These data revealed a significant dose-response relationship between sitting time and both all-cause and cardiovascular disease mortality. Intriguingly, When participants were stratified by activity this dose-response relationship was slightly attenuated, but persisted even among those meeting PA guidelines (93) Due to these and other similar revelations, a new field of inactivity physiology was born. The main contention of this field is that inactivity is harmful in that it not only impacts typical exercise induced health benefits but prompt a set of completely divergent health implications that arise not from the lack of exercise but from the effects of inactivity (64, 147).

Exercise and Postprandial Metabolism

Physical inactivity has been recognized as a major independent risk factor in the development of CVD (28). Thus, it stands to reason that one remedy for the rise of CVD in modern culture would be engaging in regular exercise. Exercise is universally recognized to decrease the risk of a variety of chronic diseases and metabolic disorders

(154). Exercise training has been shown to reduce risk of atherosclerotic plaque formation associated with excessive blood lipid concentrations (43),which leads to lower cardiovascular disease (CVD) risk in individuals who are physically active compared to inactive individuals (14). More specifically, a single bout of exercise can have impact on PPL by affecting circulating triglyceride levels following a meal (46, 76, 120, 168, 222). Studies using an intravenous lipid tolerance test indicate that the protective effect of an intense bout of exercise is attributed to accelerated TG clearance from the circulation (165)

This effect of exercise is fairly robust. Prior exercise attenuates PPL when a meal is given several hours after exercise (i.e.12 h) regardless of whether the meal is of moderate or high fat (85), or exercise is of low, moderate, or high intensity (129, 183, 186) and even in response to resistance exercise (186, 219). There are several variables that can influence the exerted effects of exercise such as timing of exercise, intensity, and energy balance as well as the composition of the test meal.

Timing of the exercise seems to be an important factor in assessing this effect. It is important to understand when a bout of exercise begins to exert a sizable effect on postprandial metabolism and when that effect subsides. Once these are correctly understood, it becomes easier to sustain healthy plasma triglyceride levels and ward off atherosclerosis and associated CVD.

It seems that the delayed effect (>12h) of exercise on PPL is more robust than the acute effect (222). When test meals are given immediately following exercise or closely there after (<4h) some mixed results have been reported (124). During this time many

factors can confound the effect of exercise such as the flux in fasting triglyceride levels from the resultant increase in lipolysis and subsequent increase in free fatty acid (FFA) delivery to the liver (175). Some of these studies show discernable difference in PPL in response to a high-fat meal, but these differences could not be seen if a moderately-fat test meal was employed (155, 158). The lack of a difference persists in cases where either moderate or low intensity exercise was undertaken (155, 156). Also during this time, very-low intensity (25-30% VO_{2max}) and resistance exercise have shown no effect, or even adverse effects on PPL (23, 92).

Conversely, when a test meal is given 12-16 hours after an exercise bout the effects are much more ubiquitous on PPL. The effectiveness of exercise is apparent over a vast range of intensities from 25-90% of maximal oxygen uptake (VO_{2max}), and durations of exercise ranging 30-120 minutes (52, 76, 120, 130, 168, 183, 222). This is also true regarding the mode and type of exercise. As previously mentioned, aerobic and resistance training both show clearly discernable benefits on PPL. High intensity interval training (HIIT) has also been employed and seems to show an even greater ability to attenuate PPL (47, 183)

However, this attenuation does not continue indefinitely. Data suggest this effect of acute exercise is transient in nature and may only continue to show appreciable effects on PPL for 24-40 hours (76, 124, 187). It seems that while this attenuated PPL response is still distinct from non-exercising controls up to 42 hours post exercise, (75, 76) but this difference is no longer apparent after 60 hours (76, 77). Therefore despite evidence that

active middle-aged men have lower plasma triglyceride concentrations than their sedentary counterparts, both fasting (83) and in response to a high-fat meal (164), this training provides no protective effect after the transient effects of the last bout subsides in 42-60 h. This has been seen in investigations ranging from 1 week of training to 6 months. After one week of training investigators report improved postprandial metabolism in the absence of any other metabolic changes, suggesting the improvements are a transient and induced by activity in the final bout of exercise (159). When participants are asked to abstain from exercise for 60 h prior to measuring PPL, the difference between active (endurance and sprint/strength trained) and inactive counterparts is abolished (185). Interventional studies have shown that one month of aerobic training appears ineffective in positively influencing PPL, when it is measured 2 (53, 169), 2.5 (77), or 9 days (7) after detraining.

Intensity is also an important factor in modulating this PPL response. A bout of high intensity interval aerobic exercise or resistance exercise may reduce fasting plasma triglycerides the next day in a similar magnitude and via a similar mechanism as moderate intensity aerobic exercise of almost twice the energy expenditure (24, 67). Furthermore, HIIT training has been shown to induce a larger reduction in the incremental area under the curve (iAUC) response than aerobic (46, 184). Freese et al. (47) found that an accumulation of 18 minutes of HIIT could induce an attenuation of PPL similar to continuous aerobic exercise lasting 30 minutes or longer. Further these authors compared 30 minutes of brisk walking to five, 30 second maximal sprints with 4 minutes of rest and found that only the HIIT sprints caused a reduction in PPL

incremental AUC (48). Some contend that this method is flawed in the inability to accurately account for total energy expenditure because of the anaerobic component of this type of exercise and subsequent increase post-exercise oxygen consumption (46). However, when Trombold et al. (183) compared 2 min intervals at 90% of VO_{2peak} to 1 hour of continuous exercise at 50% of VO_{2peak} resulting in the same energy expenditure, these investigators found the intervals significantly reduced PPL compared to control and continuous exercise. The results indicated HIIT exercise was better at attenuating PPL, as the investigators found a significantly reduced triglyceride incremental AUC when compared to the continuous exercise and non-exercise groups (183). Nevertheless, the same group produced data indicating that intensities as low as 25% VO_{2max} still produced significant differences in PPL compared with controls (99). Cumulatively these data suggest that while a large range of intensities can be employed to combat PPL, increased intensities do, in fact, result in more effective and potent reductions.

It has been suggested that this most crucial variable in permitting an attenuation of PPL is not the intensity or duration of exercise but the existence of an energy deficit resulting from the exercise undertaken (8, 46, 124, 129, 178). Under this premise, Gill and Hardman (52) investigated energy deficits induced by exercise and reduced caloric intake. They found caloric restriction produced positive results but the deficit imposed via exercise was superior at reducing PPL compared with equivalent deficits resulting from caloric restriction. However, aerobic exercise has been shown to lower plasma triglyceride concentrations in the absence of concomitant energy deficit (2). Tolfrey et al.

(179) found that high intensity cycling can reduce PPL, even when energy expenditure is replaced. However, a still greater effect existed in a group which remained hypocaloric, suggesting an interaction.

One prevailing hypothesis suggests that the mechanism by which these exercise-induced improvements in postprandial metabolism are achieved is via an increase in lipoprotein lipase (LPL) activity within the active skeletal muscle (46, 124). This is supported by studies suggesting the exercise-induced attenuation of PPL cannot be attributed to acute exercise-induced changes in blood flow or energy stores (225). Skeletal muscle LPL, found on the vascular endothelium, is the main site of triglyceride removal, and the activity of LPL can be increased through exercise (58, 164, 165). It seems contractions of the skeletal muscle cause a transient (165), tissue specific (164) increase in skeletal muscle LPL enzyme activity. This increase in LPL activity, as well as protein, is a local, delayed response to contractile activity and is independent of catecholamine and other cardiometabolic responses to exercise (58). The exercise-induced increase in LPL mRNA levels peaks 4 h after exercise, whereas LPL protein peaks 8 h after exercise and returns to baseline values within 24 h post-exercise (97, 165). While exercise has a robust effect on LPL, inactivity may have an even greater effect. Over 90% of LPL activity typically present in skeletal muscle can be lost by preventing ambulatory activity, while light activity has been shown to increase LPL activity (15). In fact, Bey and Hamilton (15) reported that intensities roughly equivalent to casual walking could maximally activate LPL in slow muscle fibers.

It seems that elevated plasma insulin concentrations can suppress LPL activity

(97). Three days of high carbohydrate (CHO) diet results in increased plasma insulin and 55% lower LPL activity (89). Conversely, a low-CHO diet for the same duration results in an increase in LPL activity (115). This may serve to reconcile some of the spurious results found in previous research. Even if the same exercise was employed the carbohydrate content of the test meal could results in divergent responses. To address this, Trombold et al (184) used a high (83%) and low CHO (12.5%) test meal following identical exercise regimens. In this study, although both replaced the exercise-induced calorie deficit, only the group given the low-CHO test meal showed reductions in PPL. It stands to reason that an exaggerated rise in plasma insulin could cause a subsequent suppression of LPL activity in response to the high carbohydrate content of the test meal. Nevertheless, exercise-induced reductions in PPL have also been documented even in the absence of significant changes in plasma insulin or improvements in insulin sensitivity (54, 123, 129).

Alterations in Ambulatory Activity

It is well established in the current literature that regular physical activity, including exercise, is advantageous for those seeking to reduce risk of detrimental health outcomes (68). Exercise is different from physical activity by its purposive nature (25). While the dramatic effects of the various types and modes of exercise have dominated the literature, more recently the effects of changing regular non-exercise physical activity

have gained a greater appreciation and considered more frequently in interventional studies.

Light intensity activity has an inverse linear relationship with a number of cardio-metabolic markers and the impact of these activities as a biological stimulus contributing to better health has probably been significantly underestimated (36, 72). Much of this is attributed to the replacement of unhealthy behaviors with healthy ones. In fact, almost all variation in sedentary time across the population is related to the extent to which sedentary time is replaced by light intensity activity (36)

One of the most attractive and practical interventions currently used in investigations of the effects of physical inactivity is reductions in daily step number. This is because reductions in daily steps are less extreme than bedrest, spaceflight, and other models of inactivity. Moreover, using easily accessible pedometers and accelerometers makes monitoring much less arduous and more replicable. Due to the tangible nature of a daily step number metric and the ability to employ these interventions under free-living conditions, the conclusions garnered from these studies are more readily applicable to a general population. This is also reasonable in view of growing epidemiological evidence that suggest increased daily walking, which is the largest component of daily physical activity (113, 114), is associated with decreased risk of cardiovascular events (122). Conversely, decreased walking has been shown to have a number of adverse health effects such as insulin resistance (109, 145) and obesity (114).

Several recommendations have been given as to the number of steps individuals should accrue each day. Many cite a 10,000 step daily goal as a benchmark to improve

health, although this seems to be a rather arbitrary number that finds its origins nearly 60 years ago in Japanese health clubs and pedometer promotions (189). Further studies on this topic have focused on empirically-driven data to craft a daily step goal (119, 188). Tudor-Locke et al (195) and others (161) have found that, when translating the current PA guidelines into a standard daily step number, approximately 8,000 steps/day was consistent with obtaining the recommended 30 minutes/day of MVPA.

While these studies are considered less extreme than studies of extended bed-rest and the like, they are still quite potent with relatively short interventions exerting sizable effects. Some authors have found reducing daily step numbers from >10,000 to <2,000 steps/day in participants that VO_{2max} decreased ~7% in just two weeks (108, 109). These data are underscored by the assertion made by Trappe et al (180) that the decline in cardiorespiratory fitness from ages 30 to 50 is due almost exclusively to the increase in physical inactivity. Further, as individuals age daily step counts decrease (190, 192, 215) even as daily walking makes up a greater portion of an individual's total physical activity (182, 193).

Furthermore, reduced ambulatory activity in durations shorter than a week have been shown to impair insulin sensitivity and elevated glucose responses to oral glucose tolerance tests (OGTT) (108, 109, 128, 145). By experimentally reducing daily step number for 1 week from ~10,500 to ~1,500 Olsen et al. (145) reported an increase of more than 52% in the area under the curve of plasma insulin during an (OGTT) with the potential to grow to nearly 80% greater, if the reductions were maintained for 2 more

weeks (145). This is due to a decrease in insulin sensitivity and increases in insulin and c-peptide in response to an OGTT within 3 days of reduced ambulatory activity. Due to these changes, development of type 2 diabetes and metabolic syndrome become more prevalent in inactive individuals. In an analysis of 2,500 participants Vander Berg et al (197) found that for each additional hour of sitting or lying during the waking hours odds of developing type 2 diabetes increased 22% and 39% for development of metabolic syndrome. Interestingly these elevated odds were independent of participation in high-intensity physical activity. Much less work has been done to directly investigate the effect of decreasing daily steps and PPL responses. However, a two-week reduction in steps has been associated with an increase of 27% in postprandial plasma triglyceride AUC (109, 145). This indicates the effects may be similar to those on glucose metabolism.

Exercise Resistance

Recently an alarming phenomenon of 'exercise resistance' has been postulated by some in response to inactivity (1, 22, 38, 98). Relatively few studies have considered the effects of inactivity on metabolism in conjunction with acute and chronic exercise. This type of design is pertinent in a culture where individuals are able to achieve physical activity guidelines (i.e. a 30-minute brisk walk) and sit for 15 hours or more in the same day (64, 65). Epidemiologists have begun to recognize an alarming trend, identifying a subset of those classified as "physically active" are not fully realizing the protective effect of that activity (146). It seems that physical inactivity may cause the production of some unknown factors that impair normally healthy physiological stimuli, such as

exercise, from occurring or being realized. Or, alternatively, the adverse effects of a physically inactive lifestyle may be independent of the protective effects normally associated with exercise (36, 65, 146).

A plausible explanation to this trend arose in 2016 when the term exercise resistance was coined in a recent paper from Kim et al. (98). In this study it was found that participants that sat for ~14 hours (<1700 steps) in their waking day did not respond, by attenuating the 6-hour PPL excursion, the morning after 1-hour of running at 63% of VO_{2max},. Yet, participants in a group that were not sitting to such an extent did improve PPL the morning after the acute exercise. Interestingly, this non-response was observed in groups of both eucaloric and hypercaloric energy balance (98). While this was a remarkable observation, the study was not designed to make definitive statements on 'exercise resistance', as it did not include a non-exercise control group. In order to address this, a follow up study by Akins et al. (1) was conducted to test the existence of this phenomenon. Using a similar, randomized, cross-over design, this study employed inactivity to slightly lesser extent (~13.5h/day sitting and ~3600 steps/day) but provided an adequate control in which one of the two trials entailed prolonged sitting without exercise on the day before the HFTT. Following the 1h of exercise, the participants in this study showed no significant improvement in PPL, glucose or insulin excursions over 6 hours compared to the non-exercise control group (1). Thus, they appeared resistant to improving PPL as a results of the 1h of exercise.

Cumulatively, these finding indicate that chronic inactivity abolishes the

beneficial effect of acute exercise on reducing PPL and bolstering fat oxidation. These data show that, in participants who experience prolonged periods of inactivity, an acute bout of exercise (e.g.; 1h of running) did not improve PPL. Due to this inactivity the body appears to be resistant to deriving one of the main acute health benefits of exercise; in this case attenuation of PPL.

In yet another study, Duvivier et al. (38) asked participants to undergo one of three free living conditions. The participants either sat for 14 hours/day, sat for 13 hours/day with 1-hour exercise bout interjected to replace an hour of sitting, and a condition with light physical activity that consisted of substituting 6 hours/day of sitting, with 4 hours of walking, and 2 hours of idly standing. While both light PA and exercise increased energy expenditure above sitting, neither differed from each other in total energy expenditure even though the number of steps in the low-PA group was 5 to 6 times higher (38). Interestingly, the 1-hour bout of exercise was not able to improve resting, fasting plasma triglycerides, cholesterol, or insulin concentration over sitting alone. Conversely, minimal-PA was able to improve resting, fasting plasma triglycerides and cholesterol over sitting and insulin concentration compared to the exercise group (38). Although these results did not administer a HFTT and only reported no significant effect on fasting levels the morning after exercise, it agrees with but does not prove the concept of exercise resistance.

Naturally, questions arise as to which is the likely culprit inducing this phenomenon of ‘exercise resistance’. Could it be there is something inherently harmful with the seated posture itself or, rather, is it due to a lack of contractile activity within the

muscle due to sitting? To answer this several studies have broken up prolonged periods of sitting with standing and found no discernable reductions of triglyceride iAUC (4, 74). Still, some doubted that the extent of standing employed in previous studies was sufficient to induce a significant difference. Crawford et al (31) had participants stand for 12 hours, or more, on the day prior a HFTT. In this study, the standing intervention group, displayed plasma triglyceride and insulin iAUCs that were no different than a group who sat for more than 14 hours (31). In another study (211), participants completed 40-sprint bouts of 4 sec on an inertial load ergometer. Participants either completed these bouts consecutively at the end of 8 hours of sitting, or five sprint bouts at the top of each hour over the 8 hours of sitting. While sitting time did not differ between groups, this study showed improved PPL on the following day in the individuals who spread the sprint bouts throughout the day but not in those who completed these bouts consecutively in the evening (211). The findings suggest that exercise resistance may arise if exercise is performed in the evening following a day of inactivity but can be avoided if the same level of activity is spread throughout the day. Interestingly, because daily inactivity and exercise were similar between groups, this study suggest the development of exercise resistance may be avoided with regular contraction spread throughout the waking hours. Regular contraction may be necessary to maintain healthy function and sensitivity to healthy stimuli. While the mechanisms are yet to be clearly elucidated, the observations of Lambernd et al (110) may provide insight as they observed that single muscle fibers treated with TNF-α did not show impaired insulin sensitivity if they were also contracted. It is possible that physical inactivity causes the production of some factor(s) that impair

normally healthy physiological stimuli, such as exercise, from occurring or being realized. This hypothesis agrees with the observation that people who exercise regularly, do not realize the decreased risk of cardiovascular disease or death if they also have lifestyles characterized by chronic inactivity, or that the exercise needs to be extreme in order to be protective (e.g.; 60-75 min vigorous each day)(39).

Furthermore, available evidence suggests that this 'exercise resistance' may not be an exclusive phenomenon to just postprandial metabolism. Breen et al. (22) found that reducing daily steps for two weeks, from ~6,000 steps to ~1400 steps, induced an 'anabolic resistance'. With the use of muscle biopsies, this study was able to show that the increase in myofibrillar protein synthesis, after consumption of high-grade protein, was attenuated by 26% in inactive participants compared to baseline, after 2 weeks of reducing daily step count.

In light of these new and intriguing findings the interpretation of previous studies might be reconsidered. It is possible that inactivity impairs other healthy adaptations to normally effective stimuli. While indirectly contested (131), this new evidence may provide additional insight by which the concept of non-response to acute and chronic exercise can, at least partly, be further elucidated. If these hypotheses can be generalized, it may provide an explanation for any study that found a non-response to a physiological stimulus in that it might be related to participants having a background of too much inactivity. It is possible this phenomenon may have been present in previous studies but has gone largely unrecognized due to a lack of evidence suggesting non-exercise activity may play a role in the hypothesized response. For example, Rogers et al. (159) found 7

days of aerobic exercise resulted in a significant improvement in glucose tolerance, measured as 3-hour area under the curve of plasma glucose excursion, in response to 100g oral glucose tolerance test (OGTT) compared with a non-exercising control. This improvement occurred in the absence of cardiovascular adaptation and without changes in body mass or fat content. Thus these authors concluded the changes in glucose tolerance must be due to persistent effects of the last bout of exercise. These investigators again studied subjects after 6 months of sedentary, free-living, and found that OGTT after a single bout of exercise at the same intensity and duration as that was performed in the previous intervention failed to improve plasma glucose or insulin responses following acute exercise (159). While this seemed counterintuitive to these authors at the time, recent evidence, including the emergence of exercise resistance, may shed light on a possible explanation to results that seemed perplexing by these authors own admission in 1988. It's possible that the second group of subjects were too inactive to benefit from the acute bout of exercise.

Possible Mechanisms Inducing Exercise Resistance

Although these previous studies have demonstrated that inactivity, even with moderate exercise, has deleterious effects on triglyceride and glucose tolerance (1, 38, 98), none have directly investigated the mechanisms for exercise resistance. Therefore, current understanding requires speculation. Reduced activity of muscle LPL is perhaps the most likely explanation for the impaired triglyceride clearance (63). As previously described, LPL is upregulated post exercise inducing the insertion of additional extra binding sites on

the muscle's capillary endothelium (76, 77, 91, 124). Because of the role of LPL as the rate-limiting enzyme for removing chylomicrons and VLDL triglyceride from the circulation (201), hydrolysis of triglycerides from these carriers and subsequent uptake by the muscle would therefore be delayed (55). With the lack of difference in PPL with or without exercise found in the previous studies (1, 98), it seems inactivity can impair or completely abolish the exercise-induced upregulation of LPL. In an animal model, physical inactivity modeled by hind-limb unloading, significantly reduced the in vitro activity of muscle lipoprotein lipase, and decreased the amount of heparin-released LPL and may reduce its activity by up to 90% (15, 220). This difference in activity was seen without changes in LPL mRNA. This suggests the inhibition of LPL is post-transcriptional.

One possible, post-transcriptional mechanistic explanation for this impairment could be thioredoxin-interacting protein (TXNIP). It was recently observed that 6-h of hind-limb immobilization in rats resulted in an increase in TXNIP protein expression and mRNA and a decrease in insulin-stimulated glucose uptake in the soleus muscle (94). It was speculated that this was possibly due to endocytosis and subsequent decrease in the amount of GLUT4 at the surface of the sarcolemma (94). It seems possible a similar process could affect plasma TG clearance by skeletal muscle via downregulation of LPL on capillary endothelium, either directly or indirectly through manipulation of GPIHBP1 responsible for binding and tethering LPL to the luminal surface of the capillary (12, 17). Whether the lack of contractile activity in humans that experience severe reductions in daily steps causes dramatic increases TXNIP remains to be determined. However, the previously mentioned increase in TXNIP with immobilization protocols can be

eliminated by activation of AMPK via AICAR administration (94). This adds additional credence to the hypothesis linking accumulation of TXNIP to lack of a precursor that senses energy turnover and exercise resistance. Interestingly, both TXNIP and LPL activity are sensitive to changes in contractile activity, even at low intensity such as those seen during leisurely walking (e.g. 30% VO_{2max}) (15, 63). Furthermore, AMPK activation has been shown to also upregulate LPL activity in skeletal muscle (116). Future studies are needed to test the hypothesis that AMPK activation prevents TXNIP elevation and allows a healthy increase in GLUT4 and LPL to augment the uptake of glucose and plasma triglyceride into skeletal muscle.

Future Work

While we know that exercise is beneficial for health and wellness, we are just learning that inactivity is more than the lack of exercise and it seems to be having a separate impact on health independent of exercise. This is especially true in light of emerging evidence suggesting that this exercise may not reduce the risk of developing chronic disease and premature mortality against a background of inactivity.

Future research should expand on the newfound 'exercise resistance' hypothesis and the nature of this phenomenon, determining if it extends beyond the measures of PPL. It would be increasingly useful for the medical practitioner, exercise scientist, and public health professionals to understand this and how to counteract it. If inactivity

progressively encroaches on adaptations to aerobic exercise in a 'dose-response' fashion or occurs at some threshold, further characterizing this would be invaluable to identify what minimum level of activity is necessary to fully realize the full and proper responses to acute and chronic exercise.

Furthermore, while current research is beginning to expound on the inability of exercise to improve indices of health, much of the work has focused solely on acute bouts of exercise. It is vital that data be provided to expand on 'exercise resistance' and its presence or absence in response to an accumulated training stimulus. Investigations into postprandial metabolism have shown great merit and have been shown to be sensitive to changes induced by inactivity. However, it is also important to investigate if these adverse responses, including 'exercise resistance', extend to other cardiometabolic responses to exercise training beyond PPL. In doing so, the data provided would provide substantial evidence which could aid in crafting additional and more tangible guidelines for health and wellness. The current guidelines are currently limited to generalities such as "move more, sit less" and "avoid inactivity". This seems to be a result of suffering from a lack of quantitative evidence on the effects of inactivity. It is prudent to avoid development of prescriptive standards without sufficient data to buttress them. Nevertheless, this only underscores the need for more systematic examination of varying degrees of inactivity and their effect on markers of health. While these are laudable in their intent, the lack of definitive, quantitative conclusions on inactivity relegate these guidelines to subjective interpretation and can cause disparate effects from group to group, and individual to individual.

Lastly, whether it be accumulation of TXNIP or some other co-factor within the muscle, it is important for future research to elucidate the mechanism by which ‘exercise resistance’ develops and, again, if it is limited mainly to PPL. A mechanistic understanding of this phenomenon would be of great benefit to practitioners and scientist who will be tasked with combatting this as our society continues to grow more inactive.

Appendices

APPENDIX A: METHODOLOGICAL TECHNIQUES

Oxygen Consumption

During exercise the participants breathed through a two-way non-rebreathing valve (Hans Rudolph, Kansas City, MO). Ventilation was measured via an inspiratory pneumotachometer attached to the two-way valve (Hans Rudolph, Kansas City, MO). Expired gas samples were taken from a mixing chamber which was directly connected via capillary tubing to oxygen and carbon dioxide analyzers (Applied Electrochemistry, Models S-3A/I and CD-3A, respectively). MOXUS metabolic software (Applied Electrochemistry) was then used to continuously analyze VO2 and VCO2

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) (OxiplexTS, ISS Oximeter Model 95205, Champaign, IL) was used to measure deoxygenated hemoglobin [HHb] during exercise in a thigh muscle (i.e.; vastus lateralis). NIRS analyzes the chromophores of O2Hb and HHb, which have different optical properties of absorbing near-infrared (wave length: 690 nm, 830 nm). This enables NIRS to measure the absolute concentrations of O2Hb and HHb in real-time and noninvasively (21, 57, 136).

Before every test, the NIRS was calibrated after about 30 minutes of warm-up. Figure 12 shows the description of the probe designed for skeletal muscle measurements

and this probe was used for this study. The schematic of the near-infrared light penetrating 2.0 cm, 2.5 cm, 3.0 cm, and 3.5 cm in depth from skin. The acquisition frequency of 2 Hz was used for this study. NIRS data was continuously monitored and averaged between minutes 9 and 10 of submaximal exercise tests for data analysis.

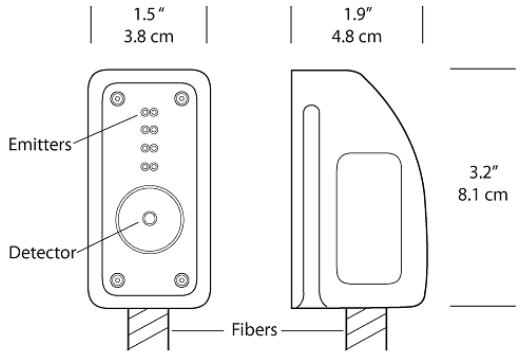


Figure 12. Diagram of OxiplexTS probe for measuring deoxygenated hemoglobin in skeletal muscle during submaximal exercise

Blood Lactate Measurements

Blood lactate concentration was determine using the following procedures and enzymatic reactions:

Part 1: Supplies, solutions, etc.

Glassware

1. Acupette capillary tubes	Qty: 2 per blood sample?
2. Eppendorf 1.5ml tube	Qty:((x+3)*2)
3. Polyproylene 12 x 75 mm test tube	Qty: (2 per blood sample)

Solutions and Reagents

1. NAD	Sigma N-7004
2. LDH	Sigma L-3916
3. Hydrazine	Sigma H-9507

4. Glycine Fisher G-46
5. Lactate Std Sigma 826-10
6. Perchloric Acid Fisher A-229 70%

PCA: to get 8%, take 57.14ml of 70% stock, bring to 500ml with dH20

Glycine-Hydrazine Buffer for 1000ml
0.33M glycine 25.02g
0.27M hydrazine 23.98mL
Mix and bring up to 1000mL with dH20, pH to 9.2

Part 2: Sample Preparation

Step 1: Prepare Reagent Cocktail

1. Prepare reagent cocktail for each sample or tube
    a. 1ml of glycine-hydrazine buffer
    b. 0.83mg of NAD
    c. 5uL of LDH, if using 1000ul/ml stock, need 5ul
2. If you have X blood samples:

. ((x+3)*2 +1) of the above cocktail recipe

a. Need the samples, one blank, two standards, all in duplicate, plus one extra so you have enough buffer for all of your samples

Step 2: Blood deproteinization

1. Protective gloves, glasses, and lab coat should be used when handling blood
2. Exactly 0.5mL of whole blood should be immediately mixed with 1.5 mL 8% PCA in Eppendorf tube
3. Vortex the tube to fully deproteinize the sample
4. Centrifuge at 4degreesC for at least 15min t 3000RPM
5. Transfer the clear supernatant to an appropriately labeled tube

a. Lactate is stable in supernatant for at least one week at 2-6degrees C, longer if frozen

Step 3: Supernatant/ Reagent Mixture

1. Add 1ml of reagent cocktail (see part 2, step 1 above)
2. Add 50uL of 8% PCA to the Eppendorf 1.5mL tubes for the blank
3. Add 50uL of two lactic acid standards to std1 and std2 Eppendorf 1.5ml tube.
4. Add 50uL of sample supernatant to sample 1 to sample N Eppendorf 1.5mL tubes.
5. Vortex each Eppendorf tube
6. Incubate tubes at 37degrees C for 45 min in shaking water bath at 60RPM

Part 3: Sample Analysis

Step 1: Spectrophotometer and Calculations

1. Warm the spectrophotometer for 30 min, read the sample at 340nM

a. Instrument: Spectrophotometer Beckmann DU-600
b. Method: A:\LAT

c. Read average time: 0.5s
d. Fixed wavelength: 340nM
e. Factor 10.13

2. Calculations

Lactate standard 40mg/100ml, 400mg/L, or 4.44mM (Sigma 826-10, now Trinity Biotech 82610)

i. Low 10mg/100mL (1.11mM)
ii. High 20mg/100mL (2.22mM)

a. Abs/E.C. = Abs/6.22
b. 1.05/0.05 = cuvette dilution (0.05mL blood in 1mL reagent cocktail)
c. Standard concentration = Abs/6.22 x cuvette dilution = Abs x 3.38
d. 3/1 = blood dilution (0.5mL blood in 1.5mL of 8% PCA)
e. Sample concentration = (Abs/6.22) x 1.05/0.05 x 3/1 = Abs x 10.13mM
f. [La] = abs x 10.13mM

Plasma Glucose Measurement

Plasma glucose we measured via spectrophotometry using commercially available kits (Pointe Scientific, Inc. Canton, USA). The plasma samples were removed from freezer (-80°C) and thawed. 5 μL of plasma sample is added to 500 μL of glucose hexokinase reagent and then incubated at room temperature for 3 minutes, following gentle mixing via a benchtop votex machine. Glucose is phosphorylated with ATP to produce glucose 6-phosphate (G-6-P) in the reaction catalyzed by hexokinase (HK). The glucose 6-phosphate is then oxidized via reduction of NAD to NADH in the reaction catalyzed by glucose 6-phosphate dehydrogenase (G6PDH). The absorbance of NADH formed was measured at 340 nm using a via spectrophotometry (Cary Eclipse Florescence Spectrophotometer, Agilent Technologies, Santa Clara, California). The concentration of NADH is directly proportional to the concentration ($mg \bullet dL^{-1}$) of glucose in the sample.

Plasma Triglyceride Measurement

Plasma triglyceride was measured via spectrophotometry using a commercially available kit (Pointe Scientific, Inc., Canton, USA). Samples were removed from freezer

(-80°C) and thawed. 3.5 µL of plasma is then pipetted off and added to 350 µL of pre-warmed (37°C) triglyceride reagent. These samples are and incubated for 30 minutes on an oscillating tray in a warm (37°C) oven. The reagent hydrolyzes triglycerides in the sample via lipase and produces glycerol and free fatty acids. Glycerol is then phosphorylated by ATP to glycerol 1- phosphate and ADP through a reaction catalyzed by glycerol kinase (GK). The glycerol 1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4- aminophenazone (4-AA) in the presence of peroxidase (POD) produces a red colored quinonimine dye. The intensity of the colored complex formed is directly proportional to the triglycerides concentration of the sample. The plate is read at 500 nm using a microplate reader (Tecan Infinite 200 PRO, Tecan Group Ltd., Männedorf, Switzerland).

APPENDIX B: RESEARCH CONSENT FORMS

Consent for Participation in Research

Title:
The Effect of Prolonged Sitting on Metabolic and Cardiovascular Responses to Short Term Exercise Training

Introduction
The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. The person performing the research will answer any of your questions. Read the information below and ask any questions you might have before deciding whether or not to take part. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study
The purpose of this study is to investigate the effect of daily sitting time on plasma triglycerides, artery function and other training adaptations resulting from 1.5 weeks of intense cycle training.

What will you be asked to do?
Before you can be admitted to the study, you will be given brief preliminary tests. This will include filling out a brief Health Research Questionnaire, and taking measurements of your height and weight. Only if you are apparently healthy and at low risk for cardiovascular disease will you be invited to participate in this study. Prior to your enrollment in the study, your peak oxygen uptake (VO_{2peak}) will be determined while exercising on a cycle ergometer (lab exercise bike) and also your heart rate during submaximal cycling will be determined.
Participation will span seventeen days, with periodic visits to the Human Performance Laboratory (HPL). You will randomly assigned to one of two groups:
Low Sitting Group: Metabolic and cardiovascular responses to exercise program and low sitting lifestyle (sitting <5h/d, >15,000 steps/d) outside of exercise.
High Sitting Group: Metabolic and cardiovascular responses to exercise program and high sitting lifestyle (sitting >11h/d, <2,500 steps/d) outside of exercise.

Step-by-Step Protocol:
Pre-Intervention Phase (Week 1)
Day 1: *High Fat Tolerance Test (HFTT) and Flow Mediated Dilation*
1. Arrival at the Human Performance Laboratory (HPL), informed consent, health history questionnaires, body mass and height.
2. Flow mediated dilation measurement.
3. Catheter insertion and fasting blood collection.
4. High fat shake consumption
5. Postprandial blood sampling hourly for 6 h (6 additional samples).
6. Expired gas collection for 20 minutes at baseline and 1, 3, 5 h after high fat shake intake.

7. Post HFTT flow mediated dilation measurement (FMD)

■ ***Total time: 430 minutes***

Day 2: *VO2peak test*

1. Arrival at HPL, body mass measurement.
2. Warm up for 5 minutes.
3. Perform peak oxygen consumption test; (VO_{2peak} test, 8-12 min.)
4. Installation of the activity monitor.

■ ***Total time: 30 minutes***

Day 3: *Submaximal Exercise test*

1. Arrival at HPL, body mass measurement.
2. Catheter insertion and baseline blood collection.
3. Warm up for 5 minutes at 50% VO2peak.
4. Perform continuous 15-minute submaximal exercise at 80% VO_{2peak} and collect blood at 15 min.
5. 5 min post exercise blood collections.

Day 4 and 5: *Low energy expenditure*

1. No Testing will be done on these days but subjects will need to be cognizant of sitting time and step count to keep it low

Day 6: *Initial training bout*

1. Arrival at HPL, body mass measurement.
2. 5-minute warm up.
3. 20-minute cycling bout at 80% $VO_{2peak.}$
4. 10-minute rest interval.
5. Two 5-minute interval exercise bouts at 95% $VO_{2peak,}$ with 5 min rest

■ ***Total time: 60 minutes***

Day 7: *High Fat Tolerance Test and Flow Mediated Dilation*
(To examine the acute effects of the single bout of exercise performed the day before).

1. Arrival at HPL, body mass and height measurement.
2. Flow mediated dilation measurement.
3. Catheter insertion and fasting blood collection.
4. High fat shake consumption.
5. Postprandial blood sampling hourly for 6 h (6 additional samples)
6. Removal of activity monitor.
7. Expired gas collection for 20 minutes at baseline and 1, 3, 5 h after high fat shake intake.
8. Post HFTT flow mediated dilation measurement.
9. Reattachment of activity monitor.

■ ***Total time: 420 minutes***

Training Phase (Week 2)

Day 1, 3 , 5 & 7: *Training Days*

1. Arrival at HPL, body mass measurement.
2. 5-minute warm up.
3. 20-minute cycling bout at 80% $VO_{2peak.}$
4. 10-minute rest interval.
5. Two 5-minute interval exercise bouts at 95% $VO_{2peak.}$

■ ***Total time: 240 minutes (60 min each of 4 days)***

Post-Intervention Phase (Week 3)

Day 1: *High Fat Tolerance Test and Flow Mediated Dilation*

1. Arrival at HPL, body mass
2. Flow mediated dilation measurement.
3. Catheter insertion and fasting blood collection.
4. High fat shake consumption.
5. Postprandial blood sampling hourly for 6 h (6 additional samples).
6. Removal of activity monitor.
7. Expired gas collection for 20 minutes at baseline and 1, 3, 5 h after high fat shake intake.
8. Post HFTT flow mediated dilation measurement.

■ ***Total time: 420 minutes***

Day 2: *Post-training submaximal exercise test*

1. Arrival at HPL, body mass measurement.
2. Catheter insertion and baseline blood collection.
3. Warm up for 5 minutes.
4. Perform continuous 15-minute submaximal exercise at 80% VO_{2peak} (of pretaining VO_{2peak})
5. Post exercise blood collections.

■ ***Total time: 40 minutes***

Day 3: *Post-training VO_{2peak} test.*

1. Arrival at the HPL, body mass..
2. Warm up for 5 minutes.
3. Perform continuous peak oxygen consumption test; (VO_{2peak} test, 8-12 min.)

■ ***Total time: 40 minutes***

Total time per subject for both trials is approximately 28h (1,680 minutes) over 17 days.

What are the risks involved in this study?

None of the above procedures are expected to be unduly painful or unsafe in healthy individuals. Maximal and submaximal cycling tests as well as the 30-minute training

sessions will all be performed in comfortable environmental conditions (e.g. 20 - 25 º C and relative humidity of ~ 50%). During VO_{2peak} testing, the last 2-4 minutes of the test may cause a feeling of fatigue and heavy breathing similar to performing 'high intensity interval training'. This feeling of fatigue generally subsides soon after completion (e.g. within 2-10 minutes). However, with all aerobic exercise, there is a risk of cardiovascular events. The risk of any sort of cardiovascular complication in apparently healthy (no documented CV disease) individuals is very low with no complications in 380,000 tests. Furthermore, in over 35 years involving several thousand exercise sessions, no subject in the Human Performance Lab has experienced any cardiac event. During each trial, an AED will be present and a CPR certified test administrator will be present. There is a small risk of muscular injury or muscular soreness within 24- to 48-hours post-session. To reduce these risks, a brief warm-up period will be performed.
Blood samples will be drawn during each HFTT and submaximal exercise visit via a venous catheter in the forearm or antecubital vein. A certified phlebotomist will insert all catheters. Minor discomfort may occur during the insertion of the catheter. The discomfort associated with the insertion of the catheter is similar to a venipuncture. Risks associated with placement of the catheter include bleeding, pain, swelling, bruising, infection, and thrombophlebitis. Each blood draw will be approximately 6 mL of blood or the equivalent of 162 mL for the entire experiment. This amount of blood is approximately 11 tablespoons, and less than 4% of total blood volume. After analysis, if subjects are found to have abnormally high fasting or post-prandial triglyceride levels, they will be alerted of this and advised to follow-up with their primary care physician. The activity monitor will be placed on your leg the day before study participation commences. Participants may experience a low level annoyance. The activity monitor will be detached for 30-60 min to load data to a computer. Subjects will carry a pedometer attached to their waist for a week before the initiation of the first trial for the estimation of an average daily steps and throughout the trials. It will not give participants any discomfort.
During the tests, participants may stop performing the task at any time and for any reason if he or she feels the need to do so. If participants wish to discuss the information above or any other risks participants may experience, participants may ask questions now or call the Principal Investigators.

What are the possible benefits of this study?
You will receive no direct benefit from participating in this study; however, each subject completing the study will be provided with a graphic and verbal description and explanation on their peak aerobic capacity, heart rate, blood pressure and metabolic responses both in fasted and non-fasting states in response to different prior physical activity/inactivity status.

Do you have to participate?
No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will

not affect your relationship with The University of Texas at Austin (University) in anyway.
If you would like to participate please fully read, sign, and return this form to the principal investigator of this study (Heath Burton). You will receive a copy of this form for your personal records.

Will there be any compensation?
You will not receive any type of payment participating in this study.

What if you are injured because of the study?

1. The University has no program or plan to provide treatment for research related injury or payment in the event of a medical problem. In the event of a research related injury, please contact the principal investigator.
2. The University has no program or plan for continuing medical care and/or hospitalization for research-related injuries or for financial compensation.
3. If injuries occur as a result of study activity, eligible University students may be treated at the usual level of care with the usual cost for services at the Student Health Center, but the University has no program or plans to provide payment in the event of a medical problem.

How will your privacy and confidentiality be protected if you participate in this research study?
Each subject will be assigned a unique Subject ID code. This informed consent form and the Health History Questionnaire are the only places where any personal identifying information will be recorded. These forms will be stored in a locked file cabinet. In all other cases, your data will only be identifiable by your unique code. Only the director of the laboratory (Dr. Coyle) will have access to a master list that will link your identity to your code.

Because you will be participating in this study and may do so along with other subjects in a small group, we will ask that you do not disclose names of participants in your group or any information that was discussed with other group members outside of the experimental session.

If it becomes necessary for the Institutional Review Board to review the study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate it with you, or with your participation in any study.
If you choose to participate in this study, you may be photographed or video recorded. Any photographs or video recordings will be stored securely and only the research team

will have access to the recordings. Recordings will be kept for 3 years after the research experiment has been completed and then erased.

Whom to contact with questions about the study?
Prior, during or after your participation you can contact the researcher **Heath Burton** at **(864)-940-4103** or send an email to **heath.burton@utexas.edu** for any questions or if you feel that you have been harmed.
This study has been reviewed and approved by The University Institutional Review Board and the study number is 2017-07-0074

Whom to contact with questions concerning your rights as a research participant?
For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or email at orsc@uts.cc.utexas.edu.

Participation

If you agree to participate please sign and return this form to a member of the research team.

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

Photography and video recording of your sessions is optional. However, if participants agree to be photographed or video recorded their images may also be used for professional and educational presentations not related to this research study.

_____ I agree to be **photographed and video recorded.**
_____ I do **not** want to be **photographed and video recorded.**

_____
Printed Name

_____ _____
Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____
Print Name of Person obtaining consent

_____ _____
Signature of Person Obtaining Consent Date

Consent for Participation in Research

Title:

Dose Response of Physical Inactivity on Plasma Triglyceride Elevation After a Meal.

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. The person performing the research will answer any of your questions. Read the information below and ask any questions you might have before deciding whether or not to take part. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study

The purpose of this study is to investigate the effect of two days of reduced daily stepping, and moderate exercise on plasma triglyceride elevation after a meal.

What will you be asked to do?

Before you can be admitted to the study, you will be given brief preliminary tests. This will include filling out a brief Health Research Questionnaire, and taking measurements of your height and weight. Only if you are apparently healthy and at low risk for cardiovascular disease will you be invited to participate in this study. Prior to your enrollment in the study, your maximal oxygen uptake (VO_{2max}) will be determined while running on a treadmill and also your heart rate during submaximal running will be determined.

Each trial will require five days, with periodic visits to the HPL:

Trial 1: Plasma triglyceride responses with two days of 2,500 steps per day and a single one-hour bout of exercise on the night of the fourth day.

Trial 2: Plasma triglyceride responses with two days of 5,000 steps per day and a single one-hour bout of exercise on the night of the fourth day.

Trial 3: Plasma triglyceride responses with two days of 7,500 steps per day and a single one-hour bout of exercise on the night of the fourth day.

The order of protocols will be randomized.

Step-by-Step Protocol:

Week prior to the initiation: *Health history questionnaires, familiarization, VO_{2max} test.*

5. Arrival at the Human Performance Laboratory (HPL), informed consent, health history questionnaires, body mass and height.
6. Installation of the activity monitor.
7. Perform resting gas measurement.
8. Warm up for 5 minutes on a treadmill.
9. Perform submaximal exercise test with four treadmill speeds lasting five minutes each. The intensity will approximate 40, 60, 70 and 80% of age predicted maximal heart rate.
10. Recover for ~ 15-20 minutes (re-hydrate to pre-exercise bodyweight)
11. Perform continuous maximal oxygen consumption test; (VO_{2max} test, 8-12 min.)

■ ***Total time: 150 minutes***

Trial sessions

Control Phase: Control Day 1 and 2 (C1 and C2)

Day prior to Control day 1: Activity monitor installation

1. Arrival at the laboratory any time before 17:00 h.
2. Installation of activity monitor

- ***Total time: 30 minutes***

■ ***Total time spent during Control Phase: 30 minutes***

Intervention Phase: D1

Day 1: Reduced daily steps

1. Sitting in preferred place to accommodate step reductions (not necessarily in HPL).

Day 2: Reduced daily steps & 1-hr treadmill running

1. Sitting in preferred place to accommodate step reductions (not necessarily in HPL).
2. Arrival at HPL at 16:50 h.
3. Exercise at 65%VO_{2max} for one hour at around 17:00 h
4. Dinner provided in the laboratory.

- ***Total time: 120 min***

Day 3: High fat tolerance test and resting fat oxidation

8. Arrival at HPL, body weight, 8:00 h.
9. Catheter insertion and fasting blood collection.
10. High fat shake intake
11. Postprandial blood sampling hourly for 6 h (6 additional samples)
12. Expired gas collection for 10 minutes at 2, 4, 6 h after high fat shake intake
13. Detachment of the activity monitor

- ***Total time: 420 minutes***

■ ***Total time spent during Intervention Phase:***

- All Trials: 600-700 minutes

Total time per subject for all trials is approximately 1900 minutes

What are the risks involved in this study?

None of the above procedures are expected to be unduly painful or unsafe in healthy individuals. The maximal oxygen uptake (VO_{2max}), submaximal tests, and 1 hour of moderate exercise at 65% VO_{2max} will be performed at 20 - 25 º C and relative humidity of ~ 50%. During VO_{2max} test, only the final 2 to 4 minutes of the test is at or near maximal levels of exertion and thus accompanied by a sensation of leg fatigue and heavy breathing. This moderate feeling of fatigue will subside soon after completion (i.e.; 2-10 min). There is a very small risk that participants could experience a muscular injury, such as muscle strain. It is also possible that muscle soreness may develop 24 to 48 hours after any given testing session. To help reduce these risks, a warm up session will be mandatory prior to performing these tests. Blood samples will be drawn during each HFTT via venous catheter in an antecubital vein. A certified phlebotomist will insert the catheters. Minor discomfort may occur during the insertion of the catheter. The discomfort associated with the insertion of the catheter is similar to a venipuncture. Risks associated with placement of the catheter include bleeding, pain, swelling, bruising, infection, and thrombophlebitis. Approximately 42 ml of blood will be drawn per trial. Over the course of the entire study approximately 126 ml of blood will be drawn. This sample volume is approximately 2.3 % of the individual's total blood volume. If participants are found to have abnormally high triglyceride levels, they will be alerted of this and advised to follow-up with their primary care physician.

The risk of any sort of cardiovascular complication in apparently healthy (no documented CV disease) individuals is very low, with no complications in numerous tests. Furthermore, in over 36 years involving more than 50,000 exercise sessions, no subject in the Human Performance Lab has experienced any cardiac event. The laboratory is currently equipped with AED. A CPR certified member of the research team will be present during all testing visits in the unlikely event of an adverse reaction.

During the tests, participants may stop performing the task at any time and for any reason if he or she feels the need to do so. If participants wish to discuss the information above or any other risks participants may experience, participants may ask questions now or call the Principal Investigators.

What are the possible benefits of this study?

You will receive no direct benefit from participating in this study. However, each subject completing the study will be provided with a graphic and verbal description and explanation on their maximal aerobic capacity, heart rate, blood pressure and metabolic responses both in fasted and non-fasting states in response to different prior physical activity/inactivity status.

Do you have to participate?

No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will not affect your relationship with The University of Texas at Austin (University) in anyway.
If you would like to participate please fully read, sign, and return this form to the principal investigator of this study (Heath Burton). You will receive a copy of this form for your personal records.

Will there be any compensation?
You will not receive any type of payment participating in this study.
What if you are injured because of the study?

4. The University has no program or plan to provide treatment for research related injury or payment in the event of a medical problem. In the event of a research related injury, please contact the principal investigator.

5. The University has no program or plan for continuing medical care and/or hospitalization for research-related injuries or for financial compensation.

6. If injuries occur as a result of study activity, eligible University students may be treated at the usual level of care with the usual cost for services at the Student Health Center, but the University has no program or plans to provide payment in the event of a medical problem.

How will your privacy and confidentiality be protected if you participate in this research study?
Each subject will be assigned a unique Subject ID code. This informed consent form and the Health History Questionnaire are the only places where any personal identifying information will be recorded. These forms will be stored in a locked file cabinet. In all other cases, your data will only be identifiable by your unique code. Only the director of the laboratory (Dr. Coyle) will have access to a master list that will link your identity to your code.

Because you will be participating in this study and may do so along with other subjects in a small group, we will ask that you do not disclose names of participants in your group or any information that was discussed with other group members outside of the experimental session.

If it becomes necessary for the Institutional Review Board to review the study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate it with you, or with your participation in any study.

If you choose to participate in this study, you may be photographed or video recorded. Any photographs or video recordings will be stored securely and only the research team will have access to the recordings. Recordings will be kept for 3 years after the research experiment has been completed and then erased.

Whom to contact with questions about the study?
Prior, during or after your participation you can contact the researcher **Heath Burton** at **(864)-940-4103** or send an email to **heath.burton@utexas.edu** for any questions or if you feel that you have been harmed.
This study has been reviewed and approved by The University Institutional Review Board and the study number is:
2018-08-0031

Whom to contact with questions concerning your rights as a research participant?
For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or email at orsc@uts.cc.utexas.edu.

Participation

If you agree to participate please sign and return this form to a member of the research team.

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

Photography and video recording of your sessions is optional. However, if participants agree to be photographed or video recorded their images may also be used for professional and educational presentations not related to this research study. Therefore, these may be kept indefinitely.

_____ I agree to be **photographed and video recorded.**

_____ I do **not** want to be **photographed and video recorded.**

_____
Printed Name

_____ _____
Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____
Print Name of Person obtaining consent

Signature of Person obtaining consent	Date

APPENDIX C: HEALTH HISTORY QUESTIONNAIRE

HEALTH HISTORY QUESTIONNAIRE

HUMAN PERFORMANCE LABORATORY – THE UNIVERSITY OF TEXAS

Subject ID:_____

Date of Birth (mm/dd/yy) _____

Age: _____

MALE _____ FEMALE ____

Height _____ Weight _____

HEALTH HISTORY QUESTIONNAIRE

HUMAN PERFORMANCE LABORATORY – THE UNIVERSITY OF TEXAS

Subject ID:_____

GENERAL HEALTH QUESTIONS

1. Are you taking any of the following medications on a regular basis? Y / N

(Psychotropics, Antihistamines, Asthma Meds, Aldomet, Clonidine,Anti-Depressants, Anti-Anxiety Meds)

2. Any over-the-counter meds? Y / N

If yes, explain:

3. Do you have any disability or impairment that affects physical performance? Y / N

4. Have you ever had any broken bones, surgery or injury to your lower extremities? Y/N

If yes, explain:

5. Have you had any significant medical problems within the last 10 years? Y / N

If yes, explain:

6. Do you have any drug and/or alcohol dependence? Y / N

If yes, explain:

7. Do you have any heart problems or coronary artery disease? Y / N

If yes, explain.

8. Do you have hypertension (high blood pressure)? Y / N

If yes, explain.

9. Do you have any lung or respiratory problems? Y / N

If yes, explain.

10. Do you, or have you previously had a history of blood clotting issues Y / N

If yes, explain.

11. Have you been diagnosed with diabetes? Y / N

12. Are you currently pregnant? Y / N

13. Do you smoke? Y / N

If yes, pattern.

14. Do you use alcohol? Y / N

If yes, pattern.

15. Do you use caffeine (cola, coffee, etc…)? Y / N

If yes, pattern.

16. Do you have any allergies that require medication? Y / N

If yes, explain.

17. Do you experience difficulty swallowing medications or vitamins? Y / N

If yes, explain.

18. Do you take any dietary supplements to increase your exercise performance? Y / N

If yes, what supplements so you normally take?

19. Have you been diagnosed with an obstructive disease of the gastrointestinal tract including but not limited to esophageal stricture, diverticulous, inflammatory bowel disease (IBD), peptic ulcer disease, Crohn's disease,ulcerative colitis, and previous gastro-esophageal surgery. Y / N

HAVE YOU EVER HAD ANY SIGNIFICANT SYMPTOMS ASSOCIATED

WITH EXERCISE?

1. Easy fatigability or prolonged fatigue after exercise? Y / N

If yes, explain.

2. Persistent chest pain during and/or after exercise? Y / N

If yes, explain.

3. Fainting or loss of consciousness during exercise? Y / N

If yes, explain.

4. Palpitations (rapid, irregular, or skipped heartbeats) during exercise? Y / N

5. Have you ever been told to give up sports because of a health problem? Y / N

PHYSICAL TRAINING HISTORY

How many years have you been training?

What type of physical training do you participate in?

Describe in general, the type of training you have performed for each of your years of training.

1st

2nd

3rd

4th

5th

6th

7th

8th

others

What is your personal best race time (if more than one please list distance, time and type)

_____

_____

_____

_____

_____

PLEASE GENERALLY DESCRIBE YOUR TRAINING PROGRAM DURING THE LAST 6 MONTHS

Type of training:

_____

_____

_____

Average time spent or work done (i.e.; distance):

_____

_____

_____

General Intensity:

APPENDIX D: ADDITIONAL TABLES FOR STUDY 1

Daily Steps	Day of Trial										
	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
Treatment											
High Step	13787± 1339	13961± 1110	18076± 2184	11096± 1361*	15734± 885	17913± 2341	17820± 2255	15231± 3044	18524± 2481	15852± 2045	18536± 1534
Low Step	4071± 844	3664± 489	5392± 963	2808± 170	3884± 577	4561± 1022	6414± 1182	4898± 774	6185± 782	6618± 873	3942± 792

Table 10. Average Daily Steps for both treatment groups during 11-day intervention (D4- D14). Average daily steps were significantly different between groups for each day measured ($p<0.001$).(*) significantly different from D12 and D14 within treatment group ($p<0.05$). Data are presented as M± SE.

Triglycerides	High Step		Low Step	
	AUCT	*AUCI*	*AUCT*	*AUCI*
Baseline	886.8 ± 79.6	322.9 ± 67.2	929.4 ± 73.3	295.9 ± 40.3
Acute Exercise	760.9 ± 73.7†	221.7 ±49.7*	967.5 ± 98.6	291.6 ± 66.5
Post Training	762.2 ± 65.5†	236.7 ±61.4*	890.4 ± 64.9	257.8 ± 27.0

Note: Data are presented as M±SE. (*) Significantly different from Baseline, $p<0.05$. (†) significantly different from baseline, $p<0.01$

Table 11. Total and incremental areas under the curve of plasma triglyceride concentrations during HFTTs at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). (*) Significantly different from Baseline, $p<0.05$. (†) significantly different from Baseline, $p<0.01$. All Data are reported as Mean ± SE.

Glucose	High Step		Low Step	
	AUCT	*AUCI*	*AUCT*	*AUCI*
Baseline	726.3±35.1	146.0± 38.6	704.3±26.2	145.0±31.2
Acute Exercise	701.7±19.3	148.6±28.0	758.8±58.6	180.7±47.5
Post Training	693.8±20.9	142.8±21.6	741.3±48.4	160.2±35.9

Note: Data are presented as M±SE

Table 12. Total and incremental areas under the curve of plasma glucose concentrations during HFTTs at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). All Data are reported as Mean ± SE.

High Step	Hours Postprandial						
	Baseline	H1	H2	H3	H4	H5	H6
Triglycerides (mg/dL)							
Baseline	94.0 ± 3.5	144.9 ± 6.2	163.3 ± 21.4	174.4 ± 23.2	152.4 ± 14.6	138.8 ± 9.5	131.9 ± 9.7
Acute Exercise	89.9 ± 6.6	114.4 ± 7.3†	138.9 ± 18.6*	148.6 ± 21.2*	134.7 ± 13.8	123.9 ± 10.8	111.2 ± 8.8
Post Training	87.6 ± 5.6	120.7 ± 6.8†	145.2 ± 17.9	150.7 ± 20.2	133.5 ± 11.6	116.9 ± 7.3*	102.8 ± 6.8*
Glucose (mg/dL)							
Baseline	97.4±4.1	--	127.5±8.9	--	133.3±9.2	--	107.3±7.1
Acute Exercise	92.6±4.9	--	118.9±5.0	--	129.1±9.4	--	113.0±4.8
Post Training	93.8±6.4	--	118.8±8.5	--	122.7±3.8	--	117.0±6.6

Note: Data are presented as M±SE. (*) significantly different from Baseline, $p < 0.05$. (†) significantly different from Baseline, $p < 0.01$.

Table 13. Temporal Responses of plasma triglyceride concentration for High Step treatment during HFTT at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). (*) significantly different from Baseline, $p < 0.05$. (†) significantly different from Baseline, $p < 0.01$. Data reported Mean±SE.

Low Step	Hours Postprandial						
	Baseline	H1	H2	H3	H4	H5	H6
Triglycerides (mg/dL)							
Baseline	105.6 ± 6.7	140.5 ± 7.4	167.7 ± 18.3	184.6 ± 19.9	164.7 ± 13.9	149.7 ± 12.8	138.8 ± 11.8
Acute Exercise	112.6 ± 11.0	148.5 ± 12.4	175.3 ± 22.2	191.6 ± 23.9	171.5 ± 18.0	154.5 ± 15.2	139.2 ± 15.9
Post Training	105.5 ± 7.3	140.4 ± 7.4	162.6 ± 14.5	176.6 ± 14.0	155.4 ± 16.4	140.3 ± 11.4	124.7 ± 11.0
Glucose (mg/dL)							
Baseline	93.3±3.5	--	118.2±7.1	--	126.7±8.6	--	121.2±10.5
Acute Exercise	97.8±3.9	--	135.6+12.5	--	135.5±14.7	--	118.8±8.6
Post Training	97.1±2.7	--	129.2±11.4	--	134.5±11.3	--	116.7±9.6

Note: Data are presented as M±SE.

Table 14. Temporal Responses of plasma triglyceride concentration for Low Step treatment during High Fat Tolerance Test at Baseline, following a single bout of exercise (Acute), and following 5 training bouts over the 9-days of training (Post Training). Data reported Mean±SE.

APPENDIX E: ADDITIONAL TABLES & FIGURES FOR STUDY 2

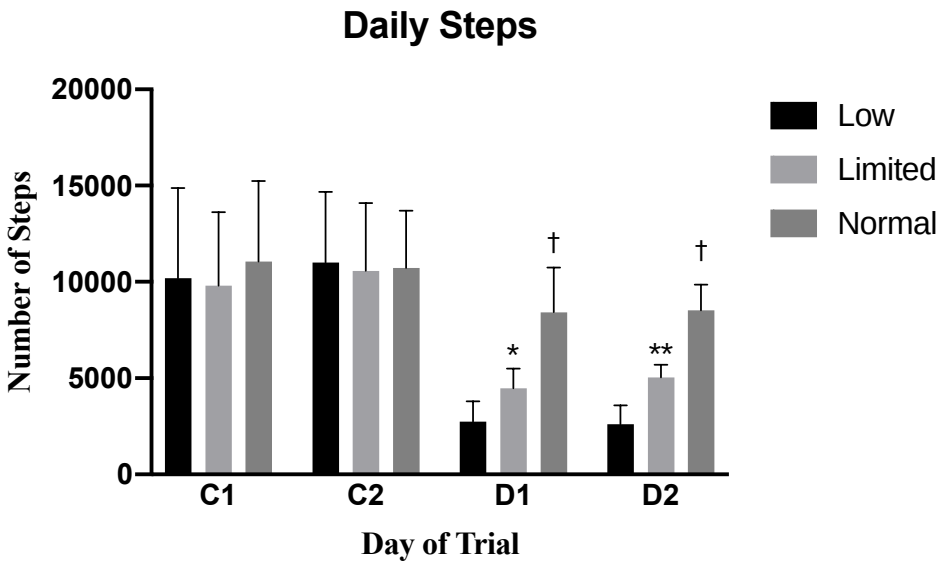


Figure 13. Average daily steps were measured via activPal activity monitor, attached on the participant's anterior thigh throughout each trial. Average daily step counts for each trial are presented for Control (C1 & C2) and Intervention Phases (D1 & D2). (*) significantly different from Low, $p<0.05$. (**) significantly different from Low, $p<0.01$. (†) significantly different from Low & Limited step trial, $p<0.05$.

Trial	AUC_T	AUC_I
Triglycerides		
Low	881.3 ± 70.7	342.3 ± 47.8
Limited	835.1 ± 73.7	348.6 ± 48.9
Normal	751.2 ± 54.6†	267.5 ± 39.2*
Glucose		
Low	709.1 ± 46.8	159.2 ± 35.2
Limited	678.7 ± 37.7	126.4 ± 25.8
Normal	683.0 ± 23.6	124.9 ± 20.6

Table 15. Total and Incremental areas under the curve of plasma triglyceride & glucose concentrations during HFTT for each trial. (*) Significantly different from Low & Limited step group, $p<0.05$. (†) Significantly different from Low step group, $p<0.01$ Data reported Mean±SE.

APPENDIX F: BIHOURLY RER MEASUREMENTS

Study 1

Pairwise comparisons (Tables 9 &10) for time points during the HFTT indicated significant differences during the second hour for RER, percent fat and percent carbohydrate oxidation.

High Step	Trial		
	Baseline	Acute	Post Training
Respiratory Exchange Ratio			
Baseline	0.760 ± 0.01	0.759 ± 0.02	0.774 ± 0.01
Hour 2	0.890 ± 0.01†	0.833 ± 0.01*	0.846 ± 0.01
Hour 4	0.819 ± 0.01	0.780 ± 0.01	0.793 ± 0.01
Hour 6	0.777 ± 0.01	0.766 ± 0.01	0.771 ± 0.01
Percent Fat Oxidation			
Baseline	80.6 ± 4.58	81.1 ± 5.87	75.7 ± 4.57
Hour 2	37.5 ± 4.71†	57.0 ± 4.13*	52.7 ± 3.71
Hour 4	61.9 ± 3.20	75.1 ± 3.57	72.2 ± 3.68
Hour 6	76.1 ± 3.39	80.0 ± 3.32	77.0 ± 2.87
Percent CHO Oxidation			
Baseline	19.4 ± 4.58	18.9 ± 5.87	24.3 ± 4.57
Hour 2	62.5 ± 4.71†	43.0 ± 4.13*	47.3 ± 3.71
Hour 4	38.1 ± 3.20	24.9 ± 3.57	27.8 ± 3.68
Hour 6	23.9 ± 3.39	20.0 ± 3.32	23.0 ± 2.87

Note: Data are presented as M±SE

Table 16. Average postprandial substrate oxidation at each measurement for HS Treatment group. (*) Significantly different from Baseline, ($p<0.05$). All Data are reported as Mean ± SE.

Low Step	Trial		
	Baseline	Acute	Post Training
Respiratory Exchange Ratio			
Baseline	0.773 ± 0.01	0.754 ± 0.01	0.760 ± 0.02
Hour 2	0.879 ± 0.01	0.879 ± 0.01	0.861 ± 0.01
Hour 4	0.826 ± 0.02	0.793 ± 0.1	0.814 ± 0.01
Hour 6	0.789 ± 0.02	0.779 ± 0.01	0.763 ± 0.01
Percent Fat Oxidation			
Baseline	76.2 ± 4.44	86.9 ± 3.19	80.6 ± 5.14
Hour 2	41.4 ± 3.83	41.4 ± 2.52	47.3 ± 3.02
Hour 4	59.5 ± 5.26	70.7 ± 4.06	68.3 ± 6.74
Hour 6	72.2 ± 5.39	75.6 ± 4.91	76.1 ± 2.95
Percent CHO Oxidation			
Baseline	23.8 ± 4.44	13.1 ± 3.19	19.4 ± 5.14
Hour 2	58.6 ± 3.83	58.6 ± 2.52	52.7 ± 3.02
Hour 4	40.5 ± 5.26	29.3 ± 4.06	31.7 ± 6.74
Hour 6	27.8 ± 5.39	24.4 ± 4.91	23.9 ± 2.95

Note: Data are presented as M±SE

Table 17. Average postprandial substrate oxidation at each measurement for LS Treatment group. All Data are reported as Mean ± SE.

Study 2

Postprandial Measurements	Trial		
	Low	Limited	Normal
Respiratory Exchange Ratio			
Baseline	0.782 ± 0.010	0.750 ± 0.007	0.739 ± 0.006
Hour 2	0.844 ± 0.018	0.833 ± 0.014	0.793 ± 0.011*
Hour 4	0.789 ± 0.016	0.781 ± 0.019	0.764 ± 0.015
Hour 6	0.776 ± 0.015	0.755 ± 0.012	0.738 ± 0.012
Percent CHO Oxidation			
Baseline	25.60 ± 3.52	14.74 ± 2.23	11.95 ± 1.77
Hour 2	46.89 ± 6.13	43.14 ± 4.70	29.39 ± 3.89*
Hour 4	28.05 ± 5.58	25.12 ± 6.49	18.09 ± 5.48
Hour 6	23.62 ± 5.09	16.52 ± 4.17	11.26 ± 4.16
Percent Fat Oxidation			
Baseline	74.40 ± 3.52	85.26 ± 2.23	88.05 ± 1.77
Hour 2	53.11 ± 6.13	56.86 ± 4.70	70.61 ± 3.89*
Hour 4	71.95 ± 5.58	74.88 ± 6.49	81.91 ± 5.48
Hour 6	76.38 ± 5.09	83.48 ± 4.18	88.74 ± 4.16

Note: Data are presented as M±SE (*) significantly different from both Low & Limited, $p<0.05$.

Table 18. Average postprandial substrate oxidation at each measurement for each trial. (*) significantly different from both Low & Limited, $p<0.05$. All Data are reported as Mean ± SE.

APPENDIX G: STUDY 1 INDIVIDUAL DATA TABLES

Biographical and VO2peak Data

LS	Age	Height	Mass	VO2peak (ml/min)		VO2peak (ml/kg/min)	
				Pre	Post	Pre	Post
1	20	157.5	71.3	2364	2409	33.2	33.2
2	21	168.9	74.2	2864	3320	38.6	45.2
3	24	162.6	70.8	1547	1719	21.9	24.0
4	21	172.7	79.9	2684	2986	33.6	37.0
5	26	177.8	68.5	3242	3376	47.3	50.3
6	21	162.6	62.2	1792	1788	28.8	28.7
7	32	157.5	59.1	1431	1449	24.2	24.7
8	25	177.8	94.9	2877	3089	30.3	33.0

HS	Age	Height	Mass	VO2peak (ml/min)		VO2peak (ml/kg/min)	
				Pre	Post	Pre	Post
1	19	154.9	60.6	2321	2594	38.3	41.4
2	35	167.6	94.1	2419	2569	25.7	27.6
3	21	175.3	90.2	3875	3888	43.0	43.4
4	26	167.6	91.25	2459	2517	26.9	28.3
5	19	167.6	69.6	3126	3560	44.9	50.7
6	18	177.8	68.25	2942	3098	43.1	46.1
7	26	157.5	45.5	1312	1596	28.8	35.4
8	23	162.6	75.9	1659	1746	21.9	22.6

Submaximal Exercise Data

LS	Work Rate (W)	VO2	%VO2peak		Heart Rate		Blood Lactate		Rating of Perceived Exertion	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	115	1834	77.6	76.1	184	182	7.4	7.5	16	14
2	164	2356	82.2	71	180	173	7.5	8.1	17	13
3	75	1269	82.0	73.8	183	184	5.8	5.1	14	15
4	148	2147	80.0	71.9	196	186	6.3	6.2	17	15
5	175	2482	76.6	73.5	187	179	6.6	6.3	16	12
6	91	1433	79.9	80.2	198	182	7.6	7.7	16	15
7	70	1153	80.6	79.5	167	169	8.8	8.8	14	14
8	167	2306	80.1	74.6	152	151	7.8	7.8	16	18

HS	Work Rate (W)	VO2	%VO2peak		Heart Rate		Blood Lactate		Rating of Perceived Exertion	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	119	1804	77.7	69.5	185	177	7.8	6.5	16	14
2	132	1915	79.1	74.5	182	171	6.3	4.1	17	12
3	222	3086	79.6	79.4	178	170	8.7	7.8	17	16
4	130	1808	73.5	71.8	156	151	5.7	5.0	14	13
5	176	2504	80.1	70.3	200	189	11.2	10.1	17	16
6	165	2373	80.7	76.6	194	179	8.4	6.1	17	13
7	55	1010	77.0	63.3	181	156	4.3	5.7	11	13
8	82	1306	78.7	74.8	175	157	8.6	8.0	16	13

Daily Steps

Day of LS	Participant #							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
4	2489	2026	2446	4133	1576	7162	7836	4903
5	2964	2130	4484	5564	1948	2926	5314	3979
6	3821	4196	6190	3681	8420	7238	8748	840
7	2004	3012	3255	2412	2622	3008	2651	3501
8	2488	2564	4684	5281	1816	5894	5564	2783
9	3602	2943	1352	3821	8066	9902	3936	2867
10	4019	3620	11626	2013	8802	5194	9734	6305
11	5473	2751	3201	5471	7198	2056	8364	4674
12	5824	2416	8150	6882	7956	6768	8164	3325
13	5722	2003	9766	7668	8984	5384	5616	7802
14	2855	2366	1600	4105	8418	6006	3478	2709

Day of HS	Participant #							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
4	10855	13664	10756	20844	8800	15544	16002	13828
5	11226	15886	13598	15802	8436	18244	15862	12630
6	15896	15242	12584	21266	28880	24314	15578	10850
7	17888	7896	8619	7926	15811	11829	8243	10557
8	14956	12886	18286	15896	19210	17500	14996	12142
9	14502	18952	30254	15890	16150	24584	13436	9538
10	10661	11003	27204	16194	17816	26742	19022	13918
11	11285	8004	32422	12570	8994	23268	16638	8670
12	8656	15679	32974	21572	17368	18840	19002	14104
13	9984	14898	17830	16824	15850	27264	16284	7884
14	12061	15017	25292	18664	21164	21866	19322	14904

Plasma Triglyceride Concentrations

Participant #	LS TG Postprandial Time (Hours)						
	Baseline	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
Baseline							
1	66.7	107.9	108.8	107.9	101.9	84.8	85.0
2	119.5	148.1	168.5	211.3	200.0	172.0	144.0
3	118.1	157.7	188.9	204.5	195.9	176.8	157.6
4	109.3	158.5	189.4	207.9	189.2	183.5	177.9
5	99.9	125.1	131.9	151.6	162.4	146.9	131.3
6	92.1	116.5	122.5	115.6	105.6	109.0	112.4
7	122.1	149.7	158.9	201.2	179.1	180.4	181.7
8	116.8	160.4	272.6	276.9	183.5	144.3	120.3
Acute							
1	58.2	94.7	95.4	97.8	93.3	87.9	75.4
2	109.8	134.5	140.9	177.2	173.4	146.1	118.8
3	158.0	198.7	221.1	231.0	195.5	187.2	178.9
4	124.8	184.0	224.9	255.8	225.7	216.3	206.9
5	110.4	137.0	145.3	149.3	139.0	126.6	114.3
6	91.2	114.2	118.9	120.3	122.3	115.0	107.7
7	145.5	168.8	173.7	208.5	180.7	181.0	181.3
8	103.2	156.4	282.4	292.6	242.3	176.1	130.2
Post Training							
1	83.2	124.8	120.9	136.4	123.1	110.6	77.4
2	83.2	139.5	177.5	150.8	110.2	114.8	119.4
3	122.2	155.5	170.5	211.3	210.0	186.4	162.8
4	128.0	172.2	198.2	208.1	201.9	162.8	123.6
5	93.1	118.2	125.0	155.6	121.8	119.0	116.3
6	86.6	111.0	117.0	141.1	100.1	103.5	106.9
7	122.3	149.8	159.1	164.8	172.3	173.9	175.6
8	125.7	151.9	232.6	245.0	203.7	151.2	115.7

Participant #	HS TG Postprandial Time (Hours)						
	Baseline	H1	H2	H3	H4	H5	H6
Baseline							
1	78.0	133.2	145.5	157.8	133.9	120.5	125.5
2	109.2	185.6	311.9	335.6	250.4	202.5	195.2
3	99.3	139.4	139.6	139.5	127.8	128.1	114.4
4	101.8	152.8	158.0	159.0	146.0	133.5	118.9
5	96.6	138.4	136.5	149.2	148.9	144.9	142.8
6	85.3	134.5	130.5	145.8	125.5	126.8	130.1
7	90.1	136.7	139.9	146.0	129.6	121.3	113.0
8	91.5	139.0	144.9	162.5	156.8	132.9	114.9
Acute							
1	56.2	91.1	102.1	105.1	84.2	77.8	75.0
2	109.5	148.5	260.7	290.6	199.5	157.3	125.0
3	82.3	103.3	110.3	114.6	102.7	115.1	111.2
4	104.2	111.9	122.4	129.0	139.4	120.5	113.9
5	104.9	132.8	143.7	151.5	153.7	140.1	132.1
6	96.5	104.3	114.4	124.8	123.1	115.9	104.7
7	71.3	93.5	103.1	117.0	102.4	94.9	79.4
8	94.1	129.9	154.4	156.4	172.3	169.4	148.0
Post Training							
1	57.7	91.5	104.7	75.3	77.0	75.6	66.3
2	79.9	153.8	261.9	276.0	192.4	149.7	127.9
3	83.9	105.0	108.8	121.8	110.3	108.8	106.3
4	93.5	127.9	138.7	159.7	134.5	123.4	117.2
5	111.9	137.4	161.5	155.1	147.2	124.5	109.1
6	100.4	119.8	136.6	142.6	132.6	119.0	110.5
7	87.7	113.0	120.0	142.0	127.4	112.1	84.4
8	85.9	117.6	129.4	133.2	146.2	122.4	100.4

Plasma Glucose Concentrations

Participant #	LS Glucose Postprandial Time (Hours)			
	Baseline	**H2**	**H4**	**H6**
Baseline				
1	92.7	126.9	169.7	132.5
2	93.6	97.2	112.1	88.7
3	77.7	160.4	95.7	92.5
4	83.0	116.2	114.5	166.5
5	101.8	105.7	121.9	96.5
6	109.8	127.6	144.9	122.0
7	96.1	106.0	110.1	112.6
8	91.7	105.5	144.7	158.5
Acute				
1	90.9	131.8	158.6	143.6
2	93.1	103.1	119.0	90.4
3	99.3	131.4	123.2	112.4
4	87.0	121.9	122.4	134.5
5	101.3	112.1	129.4	103.1
6	116.2	203.4	228.5	160.2
7	110.4	106.8	98.7	105.7
8	84.5	174.4	104.1	100.6
Post Training				
1	91.0	121.0	178.3	128.1
2	88.8	93.0	90.3	82.1
3	95.8	106.5	121.2	145.1
4	100.5	140.6	144.9	159.6
5	89.0	104.1	98.2	89.3
6	109.5	196.2	175.6	104.8
7	96.1	129.5	133.9	102.1
8	106.1	142.9	134.0	122.2

Participant #	HS Glucose Postprandial Time (Hours)			
	Baseline	H2	H4	H6
Baseline				
1	103.3	124.3	125.4	107.9
2	103.2	111.2	122.6	99.7
3	86.5	101.8	115.2	97.1
4	104.8	111.8	121.9	120.2
5	114.9	176.0	125.6	91.2
6	94.2	155.5	187.4	147.9
7	78.3	124.9	157.6	84.0
8	94.2	114.9	110.7	110.2
Acute				
1	89.5	112.4	122.1	108.8
2	98.1	112.5	184.9	92.8
3	83.1	94.1	99.8	130.9
4	100.2	120.1	123.3	113.1
5	104.5	142.9	101.4	97.3
6	106.4	128.3	137.2	112.7
7	64.2	123.9	134.9	128.3
8	95.1	117.4	129.5	120.2
Post Training				
1	76.1	81.4	131.9	121.4
2	95.8	151.9	118.3	113.2
3	70.0	93.3	124.4	81.3
4	98.8	132.2	120.8	123.9
5	125.5	143.6	101.1	107.8
6	90.0	120.6	134.7	123.6
7	84.4	111.6	131.4	148.1
8	110.1	115.5	119.1	116.6

RER Data

Participant #	LS RER Postprandial Time (Hours)			
	Baseline	**H2**	**H4**	**H6**
Baseline				
1	0.78	0.9	0.88	0.86
2	0.82	0.85	0.88	0.81
3	0.8	0.87	0.79	0.77
4	0.72	0.93	0.83	0.81
5	0.75	0.85	0.78	0.77
6	0.79	0.89	0.82	0.73
7	0.75	0.86	0.8	0.77
Acute				
1	0.77	0.88	0.83	0.84
2	0.78	0.87	0.81	0.82
3	0.72	0.9	0.8	0.76
4	0.72	0.9	0.8	0.76
5	0.75	0.85	0.78	0.77
6	0.79	0.89	0.73	0.73
7	0.75	0.86	0.8	0.77
Post Training				
1	0.81	0.9	0.89	0.77
2	0.77	0.88	0.8	0.78
3	0.72	0.86	0.79	0.74
4	0.78	0.84	0.8	0.79
5	0.73	0.86	0.81	0.76
6	0.71	0.86	0.81	0.71
7	0.8	0.83	0.8	0.79

Participant #	HS RER Postprandial Time (Hours)			
	Baseline	H2	H4	H6
Baseline				
1	0.76	0.89	0.79	0.77
2	0.79	0.91	0.85	0.81
3	0.74	0.92	0.84	0.8
4	0.71	0.88	0.79	0.77
5	0.81	0.94	0.83	0.79
6	0.78	0.84	0.8	0.77
7	0.73	0.85	0.83	0.73
Acute				
1	0.8	0.83	0.81	0.78
2	0.77	0.89	0.81	0.75
3	0.73	0.82	0.75	0.72
4	0.71	0.85	0.77	0.76
5	0.83	0.79	0.79	0.8
6	0.71	0.84	0.79	0.77
7	0.76	0.81	0.74	0.78
Post Training				
1	0.77	0.8	0.79	0.74
2	0.77	0.84	0.8	0.79
3	0.83	0.87	0.76	0.81
4	0.77	0.85	0.8	0.77
5	0.81	0.89	0.81	0.76
6	0.74	0.84	0.77	0.75
7	0.73	0.83	0.82	0.78

Postprandial Fat Oxidation **(kcal/6h)**

High Step

	1	2	3	4	5	6	7
Baseline	282.3	252.2	284.7	362.4	280.2	382.1	327.1
Acute	312.2	277.8	446.2	444.4	433.8	388.8	385.4
Post Training	403.9	295.3	360.1	385.3	372.6	427.2	337.6

Low Step

	1	2	3	4	5	6	7
Baseline	248.8	261.0	430.0	187.0	420.3	419.0	346.7
Acute	245.8	269.7	312.8	275.2	548.1	474.7	336.2
Post Training	298.2	287.2	418.1	299.0	491.1	305.6	355.8

APPENDIX H: STUDY 2 INDIVIDUAL DATA TABLES

Biographical & Exercise Data

Participant #	Age	Height	Mass	VO2max (ml/min)	VO2max (ml/kg/min)	Exercise VO_2	%VO2max	Heart Rate	RPE	Speed
1	20	71	94.1	3834	40.7	2438	63.6	134	12	4.3
2	22	71	86.4	3999	46.3	2599	64.8	172	13	5.5
3	32	61	54.6	1980	36.3	1287	65.2	139	10	4.4
4	24	73	75.7	3233	42.7	2102	61.9	161	10	4.8
5	21	62	73.5	2880	39.2	1868	64.8	166	11	4.6
6	36	73	116.2	4658	40.1	3022	64.9	140	11	4.6
7	32	67	84.1	3450	41.1	2284	66.2	153	12	4.5
8	27	69	68.3	3213	47.0	2010	62.6	155	11	5.1
9	19	66	66.1	2771	41.9	1896	65.2	159	13	4.1
10	24	69	78.4	4028	51.4	2597	64.5	160	11	6.2

Daily Steps

Participant #	Steps Day of Trial			
	C1	C2	D1	D2
Low				
1	5752	13302	1534	1006
2	6076	9650	3594	904
3	15844	13944	3365	3420
4	9911	9063	2812	2292
5	8891	10273	1305	3625
6	5257	5712	2476	3169
7	7805	8111	4610	3541
8	8554	10841	1599	2210
9	17886	19002	2833	3009
10	16013	10254	3316	2881
Limited				
1	4420	11826	3296	3484
2	7906	7691	3921	5904
3	10112	12248	5380	5078
4	9225	5852	4801	4732
5	10112	11540	6343	5203
6	6661	8040	4470	5240
7	7155	7440	4366	5110
8	10185	11013	3937	4993
9	16641	18145	3038	5728
10	15662	11892	5265	4896
Normal				
1	9003	7991	7853	8109
2	8114	10846	5844	5730
3	11550	14868	7578	8441
4	8841	8857	6572	7880
5	12208	11660	10571	9023
6	5855	6827	7550	7827
7	9194	7993	7191	8864
8	10777	9945	8122	9751
9	21016	15398	13856	8924
10	14003	12933	9175	10751

Plasma Triglyceride Concentrations

Participant #	TG Postprandial Time (Hours)				
	Baseline	H2	H3	H4	H6
Low					
1	109.7	148.6	169.9	209.1	172.9
2	113.1	179.4	169.7	175.4	157.8
3	86.6	122.6	130.4	146.2	112.6
4	98.6	152.3	158.7	142.8	124.0
5	48.6	99.5	151.7	172.2	104.1
6	124.5	232.1	260.1	299.6	231.3
7	57.2	87.2	194.4	164.1	94.9
8	85.6	138.0	137.1	130.5	117.6
9	87.6	131.0	134.2	125.1	91.1
10	86.9	184.8	217.5	199.9	124.2
Limited					
1	103.9	139.4	182.2	197.6	161.5
2	105.4	139.6	164.6	175.8	154.6
3	83.8	114.4	137.9	130.7	108.4
4	90.9	133.2	140.9	139.2	124.7
5	40.3	81.2	149.4	136.8	68.0
6	130.3	219.4	289.5	286.5	225.2
7	38.6	120.1	213.3	194.3	115.7
8	77.1	143.4	168.9	140.3	110.8
9	66.1	118.5	116.5	102.2	79.6
10	74.3	130.7	190.9	172.8	113.6
Normal					
1	92.0	146.3	188.9	178.7	182.9
2	111.4	147.3	155.1	156.4	134.7
3	82.3	112.4	115.4	118.3	99.0
4	83.6	129.9	134.4	121.6	118.9
5	52.1	63.7	130.2	128.5	66.3
6	89.3	175.0	221.0	214.9	168.3
7	50.7	96.8	165.4	135.6	71.2
8	75.3	127.7	127.5	106.8	93.2
9	72.7	110.8	105.2	88.9	95.4
10	90.1	143.2	169.9	166.5	126.6

Plasma Glucose Concentrations

Participant #	Postprandial Time (Hours)				
	Baseline	H2	H3	H4	H6
Low					
1	109.7	148.6	169.9	209.1	172.9
2	113.1	179.4	169.7	175.4	157.8
3	86.6	122.6	130.4	146.2	112.6
4	98.6	152.3	158.7	142.8	124.0
5	48.6	99.5	151.7	172.2	104.1
6	124.5	232.1	260.1	299.6	231.3
7	57.2	87.2	194.4	164.1	94.9
8	85.6	138.0	137.1	130.5	117.6
9	87.6	131.0	134.2	125.1	91.1
10	86.9	184.8	217.5	199.9	124.2
Limited					
1	103.9	139.4	182.2	197.6	161.5
2	105.4	139.6	164.6	175.8	154.6
3	83.8	114.4	137.9	130.7	108.4
4	90.9	133.2	140.9	139.2	124.7
5	40.3	81.2	149.4	136.8	68.0
6	130.3	219.4	289.5	286.5	225.2
7	38.6	120.1	213.3	194.3	115.7
8	77.1	143.4	168.9	140.3	110.8
9	66.1	118.5	116.5	102.2	79.6
10	74.3	130.7	190.9	172.8	113.6
Normal					
1	92.0	146.3	188.9	178.7	182.9
2	111.4	147.3	155.1	156.4	134.7
3	82.3	112.4	115.4	118.3	99.0
4	83.6	129.9	134.4	121.6	118.9
5	52.1	63.7	130.2	128.5	66.3
6	89.3	175.0	221.0	214.9	168.3
7	50.7	96.8	165.4	135.6	71.2
8	75.3	127.7	127.5	106.8	93.2
9	72.7	110.8	105.2	88.9	95.4
10	90.1	143.2	169.9	166.5	126.6

RER Data

Participant #	Postprandial Time (Hours)			
	Baseline	H2	H4	H6
Low				
1	0.829	0.815	0.791	0.784
2	0.809	0.774	0.747	0.741
3	0.721	0.771	0.756	0.733
4	0.814	0.836	0.798	0.761
5	0.786	0.87	0.748	0.77
6	0.786	0.848	0.761	0.796
7	0.794	0.799	0.851	0.762
8	0.772	0.947	0.898	0.885
9	0.748	0.891	0.79	0.721
10	0.761	0.923	0.812	0.809
Limited				
1	0.764	0.776	0.714	0.697
2	0.754	0.812	0.747	0.75
3	0.72	0.831	0.772	0.744
4	0.793	0.818	0.774	0.729
5	0.726	0.851	0.763	0.751
6	0.736	0.888	0.756	0.759
7	0.745	0.848	0.827	0.794
8	0.752	0.914	0.85	0.841
9	0.757	0.781	0.908	0.752
10	0.755	0.895	0.795	0.737
Normal				
1	0.721	0.766	0.727	0.731
2	0.724	0.777	0.791	0.718
3	0.74	0.809	0.739	0.726
4	0.743	0.789	0.741	0.723
5	0.776	0.836	0.788	0.726
6	0.717	0.755	0.757	0.735
7	0.721	0.829	0.75	0.73
8	0.745	0.844	0.732	0.735
9	0.758	0.79	0.729	0.71
10	0.748	0.736	0.886	0.844

Postprandial Fat Oxidation **(kcal/6h)**

	Participant									
Trial	1	2	3	4	5	6	7	8	9	10
Baseline	417.8	512.5	522.6	417.8	512.5	522.6	417.8	512.5	522.6	417.8
Acute	519.1	476.0	472.1	519.1	476.0	472.1	519.1	476.0	472.1	519.1
Post Training	301.7	231.0	265.5	301.7	231.0	265.5	301.7	231.0	265.5	301.7

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